Research Article

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Evaluating The Frequency of Autoantibodies in Patients with Chronic Hepatitis B

Kronik Hepatit B Hastalarında Otoantikor Sıklığının Değerlendirilmesi

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ABSTRACT

Objectives: The objective of this study is to evaluate the frequency of autoantibodies retrospectively in newly diagnosed chronic hepatitis B (CHB) patients.
Materials and Methods: The study was retrospectively conducted in between Jan 2010 and August 2015 in a research and training hospital in İstanbul with 122 patients (ages of 17-80) consulted to Gastroenterology and Infection Diseases and diagnosed with CHB and 117 healthy control group. In both groups, positive and negative rates of anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA) and anti-liver-kidney mitochondrial antibody (anti-LKM) were compared.
Results: No AMA and LKM1 were observed in any patient or control groups. ANA result was positive in 9.8% of the patient group and 8.5% of the control group; and there was no statistically significant difference between them (p>0.05). ASMA result was positive in 5.7% of the patient group and 0.9% of the control group; and the difference between them was very close to significance but statistically not enough to be significant (p>0.05). Hepatosteatosis level of the patient group was significantly lower than the control group, statistically (p<0.05).
Conclusion: In this study, it is concluded that the low frequency of autoantibody may depend on the fact that it was only examined in CHB patients who are not receiving any treatment. Examination of autoantibodies in newly diagnosed chronic hepatitis B patients before and after treatment may provide insight in terms of occuring autoimmune phenomena cases and extrahepatic findings.
Keywords: Hepatosteatosis, chronic hepatitis B, autoantibody

ÖZ

Amaç: Bu çalışmada yeni tanı konulmuş kronik hepatit B (KHB) hastalarında otoantikor sıklığını retrospektif olarak değerlendirmek amaçlanmıştır.
Gereç ve Yöntemler: Çalışma Ocak 2010-Ağustos 2015 yılları arasında, İstanbul ilindeki bir eğitim araştırma hastanesinin gastroenteroloji ve enfeksiyon hastalıkları polikliniklerine başvuran, KHB tanısı alan 17-80 yaş arası 122 hasta ve 117 sağlıklı kontrol grubu ile retrospektif olarak gerçekleştirilmiştir. Her iki grupta anti-nükleer antikor (ANA), anti-düz kas antikoru (ASMA), anti-mitokondriyal antikorlar (AMA) ve anti-karaciğer-böbrek mitokondriyal antikoru (anti-LKM) pozitiflik ve negatiflik oranları karşılaştırılmıştır.
Bulgular: Hasta ve kontrol grubundaki hiçbir olguda AMA ve LKM1 görülmemiştir. Hasta gruptaki olguların %9,8'i ve kontrol grubundaki olguların da %8,5'inde ANA sonucu pozitif olup, aralarında istatistiksel olarak anlamlı bir farklılık bulunmamıştır (p>0,05). Hasta gruptaki olguların %5,7'si ve kontrol grubundaki olguların da %0,9'unda ASMA sonucu pozitif olup; aralarındaki farklılık anlamlılığa çok yakın olmasına karşın istatistiksel olarak anlamlı bulunmamıştır (p>0,05). Hasta grubun hepatosteatoz düzeyi, kontrol grubundan istatistiksel olarak anlamlı düzeyde düşük bulunmuştur (p<0,05).
Sonuç: Bu çalışmada gerçekleştirilen araştırmalar göstermektedir ki, otoantikor sıklığının düşük olması, yalnızca tedavi almayan KHB hastalarında bakılmasına bağlı olabilmektedir. Yeni tanı alan kronik hepatit B hastalarında tedavi öncesi ve sonrası otoantikor bakılmasının; ortaya çıkabilecek otoimmün olaylar ve ekstrahepatik bulgular açısından fikir verebileceği değerlendirilmiştir.
Anahtar Kelimeler: Hepatosteatoz, kronik hepatit B, otoantikor

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Introduction

Chronic Hepatitis B (CHB) infection is an important morbidity and mortality reason worldwide. Approximately 400 million people are infected with this virus and 1 million people die every year due to the complications associated with CHB infection such as liver cirrhosis, and hepatocellular carcinoma (1,2). Hepatitis B virus (HBV) have various antigenic molecules such as 42 nm partial double-stranded deoxyribonucleic acid (DNA) molecules surrounded with core proteins, hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg). The presence of HBsAg positivity in the blood for more than 6 months is defined as chronic HBV infection. CHB infection has a wide clinical spectrum from asymptomatic carriage to fulminant liver failure. Even though it is a hepatotropic virus, many other organ systems are also affected as a result of the autoimmunity that emerged as a result of its antigenic molecules. Many previous studies have revealed these extrahepatic involvements. Among them, the most commonly known are membranous glomerulonephritis and systemic necrotizing vasculitis (3,4).

Autoantibodies are the antibodies that are produced against the proteins, nucleic acids, carbohydrates, lipids and complex molecules of an organism. They benefit not only in diagnosis but also in progression. During CHB infection, autoantibody increase is commonly seen (5). It is known that these non-specific autoantibodies develop against some antigens of HBV and as a result of the inappropriate-excessive immune system activation of the host. As a result of the cross reaction between the viral and host antigens via the molecular similarity theory, it is revealed that high autoantibody may occur in the blood and in various tissues (6). These antibodies may cause a patient infected with HBV to be misdiagnosed with autoimmune hepatitis, toxic hepatitis, or systemic lupus erythematosus. High autoantibody in the individuals infected with HBV can be comorbid with a disease or it may be associated only with HBV (7). The aim of the present study is to determine the autoantibody frequency in newly diagnosed CHB patients.

Materials and Methods

In order to conduct the study, approval was taken from the Ethics Committee of the Ministry of Health Bağcılar Training and Research Hospital (approval number: 2015/414). Each of the patients included in the study signed an "informed consent form" that provides information about the study and states that the consent of the patient is taken. In the study, autoantibody frequencies of 122 patients who applied to the departments of Internal Medicine, Gastroenterology and Infectious Diseases outpatient clinic of a training and research hospital within the boundaries of İstanbul city between January 2010 and August 2015, were aged between 17 and 80 years, and newly diagnosed with CHB were retrospectively assessed. Demographic information of the patients was recorded from the patient files. Hepatic activity index (HAI) and fibrosis levels of the patients, who underwent liver biopsy, were recorded. HBsAg, anti-HBs, HBeAg, anti-HBe, HBV-DNA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time, albumin, anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA), and anti-liver-kidney mitochondrial antibody (anti-LKM) among the laboratory parameters were recorded and the frequency was statistically detected in CHB patients. One hundred seventeen healthy individuals who applied to the internal medicine and infectious diseases outpatient clinic and had no chronic diseases were included in the control group in the study. Healthy individuals were informed about the study and their consents were obtained. AMA, ANA, ASMA and anti-LKM examinations of the control group were requested and whole abdomen ultrasonographies were taken and hepatic lipidosis grades were detected. The patients who previously received interferon or oral anti-viral therapy, used alcohol or any drugs or toxic substance that may cause autoantibody positivity, were diagnosed with CHC, delta hepatitis or any viral or bacterial infections, liver cirrhosis or hepatocellular carcinoma, were diagnosed with rheumatologic diseases that involves liver and may cause autoantibody increase such as autoimmune hepatitis, primary biliary cirrhosis, autoimmune cholangitis, primary sclerosing cholangitis, had cardiac, renal and liver failures, and were pregnant were excluded from the study. 0.3 mL serum samples of ANA, ASMA, anti-LKM and AMA kits were sent and they were studied with Immune Fluorescent Technique in the Helios Helmed Integrated Optical System (AESKU. SYSTEMS) device. Hemogram was examined via the Multiangle Polarized Scatter Separation method in the Cell Dyn Ruby (ABBOTT) device. HBsAg, anti-HBs, anti-HVC, and anti-human immunodeficiency virus were studied by the microelisa method in Microelisa Analyser (DIASORIN). Biochemical tests such as ALT, AST, albumin, creatinine, and glucose were performed via photometric method in the Roche Hitachi-Cobas C systems device. Thyroid stimulating hormone, T4, triglyceride and total cholesterol were examined using the electrochemiluminescence immunoassay method in Roche Hitachi-Cobas C systems device.

Statistical Analysis

While assessing the results obtained in the study, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for the statistical analyses. Shapiro-Wilk test was used to assess whether or not the parameters were normally distributed. In the data assessment, descriptive statistical methods (mean, standard deviation, frequency) were used. Additionally, Student's t-test was used to make comparison of normally distributed parameters between two groups in the quantitative data. Mann-Whitney U test was used to make comparison of non-normally distributed parameters between two groups. Chi-square test, Fisher's exact, chi-square test, and Yates' correction for Continuity were used for the comparison of qualitative data. Significance was assessed at the level of $p<0.05$.

Results

The study was conducted on a total of 239 cases, who were aged between 17 and 70. 97 (40.6%) of the participants were male and 142 (59.4%) were female. Mean age of the cases was 37.45±9.52 years. No statistically significant difference was found between female and male cases in the patient and control groups in terms of mean age and mean body mass index (BMI), gender distribution, smoking and alcohol use rates ($p>0.05$). (Table 1).

There was no statistically significant difference between the male and female cases in the patent group in terms of diabetes and hypertension prevalence (p>0.05). (Table 2). Anti-HBe was positive in 104 (85.2%) of those in the patient group and mean HBV-DNA level was 50495433±237566076.1, mean HAI score was 6.52±2.28, and mean fibrosis score was 1.80±1.12 (Table 3). AMA and LKM1 were not observed in any of the cases in the patient and control groups. ANA result was positive in 9.8% of the cases in the patient group and 8.5% of the cases in the control group. ASMA was positive in 5.7% of the cases in the patient group and 0.9% of the cases in the control group and no statistically significant difference was found between them (p>0.05). Mean blood glucose, AST, ALT and creatinine values of the patients were significantly higher than the control group (p<0.01; p<0.05). No significant difference was found between the groups in terms of haemoglobin, haematocrit, platelet, gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), albumin, globulin, total cholesterol, and triglyceride levels (p>0.05). It was found that ultrasonography (USG) hepatosteatosis level of the patient group was lower than the control group (p<0.01) (Table 4). No significant difference was found between the cases with positive and negative ANA and ASMA outcomes in the patient group in terms of age, BMI, USG, total cholesterol and triglyceride levels and gender distribution (p>0.05). No significant difference was found between the cases with positive and negative ANA outcomes in the control group in terms of age, BMI, USG, total cholesterol levels, and gender distributions (p>0.05). Triglyceride levels of the cases with negative ANA result were found to be significantly higher than the cases with positive ANA result (p<0.05) (Table 5).

No significant difference was observed between the HAI and fibrosis levels of the cases with positive and negative ANA and ASMA results in the patient group (p>0.05).

Table 1. Assessment of the groups in terms of demographic characteristics

	Patient (n=122)	Control (n=117)	Total (n=239)	p
Age (Mean ± SD)	37.61±8.73	37.28±10.32	37.45±9.52	[1]0.79
BMI (Mean ± SD)	27.08±4.81	27.11±5.11	27.10±4.95	[1]0.96
Male age (Mean ± SD)	38.52±8.84	38.19±11.30	38.36±10.05	[1]0.87
Male BMI (Mean ± SD)	26.74±4.31	27.64±4.44	27.17±4.37	[1]0.31
Female age (Mean ± SD)	36.97±8.66	36.67±9.65	36.82±9.13	[1]0.84
Female BMI (Mean ± SD)	27.33±5.15	26.76±5.52	27.05±5.33	[1]0.52
Gender n%				
Male	50 (41%)	47 (40.2%)	97 (40.6%)	[2]0.89
Female	72 (59%)	70 (59.8%)	142 (59.4%)	
Smoking n%	38 (31.1%)	25 (21.4%)	63 (26.4%)	[2]0.08
Alcohol n%	4 (3.3%)	2 (1.7%)	6 (2.5%)	[3]0.68

[1]Student t test, [2]chi-square test, [3]Fisher's exact test
SD: Standard deviation, BMI: Body mass index

Table 2. Assessment of diabetes and hypertension in the patient group in terms of gender

Patient group	Male (n=50)	Female (n=72)	Total (n=122)	p
	n (%)	n (%)	n (%)	
Diabetes	4 (8%)	4 (5.6%)	8 (6.6%)	0.715
Hypertension	3 (6%)	3 (4.2%)	6 (4.9%)	0.688

Fisher's exact test

Table 3. Assessment of distribution of the hepatitis B data in the patient group

Patient group		n	%
HBeAg (IU/mL)	Positive	18	14.8
	Negative	104	85.2
Anti-HBe (IU/mL)	Positive	104	85.2
	Negative	18	14.8
	Minimum	**Maximum**	**Mean ± SD (median)**
HBV-DNA (IU/mL)	1795	2100553841	50495433±237566076.1 (25340)
HAI	2	12	6.52±2.28 (6)
Fibrosis	0	6	1.80±1.12 (2)

HbeAg: Hepatitis B e antigen, HAI: Hepatic Activity index, SD: Standard deviation

HbeAg outcome was positive in 8.3% of the cases with positive ANA result and in 15.5% of the cases with negative ANA result in the patient group, and anti-HBe result was positive in 83.6% of the cases with negative ANA result and in 100% of the cases with positive ANA result and no significant difference was found between them (p>0.05). HbeAg result was positive in 15.7% of the cases with negative ASMA result and in 0% in the cases with positive ASMA result in the patient group. Anti Hbe result was positive in 84.3% of the cases with negative ASMA result and in 100% of the cases with positive ASMA result in the patient group and no significant difference was seen between them (p>0.05).

Discussion

There is a large number of evidence indicating that CHB and CHC infections may cause extrahepatic findings, autoantibody formation, and autoimmune diseases. Numerous studies have reported autoantibody frequency at different rates in the individuals infected with hepatitis C. In a study, the prevalence of autoantibodies was investigated in the CHB and CHC patients with positive HBV-DNA and HCV-RNA results who did not receive any treatment. A total of 63 patients were included in the study as 30 diagnosed with CHC and 33 diagnosed with CHB. RF was determined as 30%, ANA as 10%, and AMA as 6.7% in CHC patients; on the other

Table 4. Assessment of the autoantibody positivities, laboratory data and ultrasonography hepatosteatosis results of the groups

	Patient (n=122)	Control (n=117)	Total (n=239)	p
	n (%)	n (%)	n (%)	
AMA	0 (0%)	0 (0%)	0 (0%)	-
ANA	12 (9.8%)	10 (8.5%)	22 (9.2%)	0.90
ASMA	7 (5.7%)	1 (0.9%)	8 (3.3%)	0.06
LKM1	0 (0%)	0 (0%)	0 (0%)	-

Continuity (Yates) correction and Fisher's exact test were used.
AMA: Antimitochondrial antibody, ANA: Antinuclear antibody, ASMA: Anti-smooth muscle antibody

Table 4. Contuniued

	Patient (n=122)	Control (n=117)	Total (n=239)	p
	Mean±SD	Mean±SD	Mean±SD	
HgB (g/dL)	13.66±1.84	13.68±1.78	13.67±1.81	10.90
Htc (%)	41.84±5.3	41.44±4.93	41.64±5.11	10.53
Plt (K/mL)	243.66±65.47	256.63±58.78	250.01±62.49	10.10
Blood glucose (mg/dL) (median)	98.34±45.97 (89.5)	86.9±9.09 (86)	92.74±33.87 (88)	20.009**
AST (U/L) (median)	31.69±22.01 (27)	20.71±6 (20)	26.31±17.15 (22)	20.001**
ALT (U/L) (median)	42.45±33.72 (30.5)	22.55±14.2 (19)	32.71±27.85 (24)	20.001**
GGT (U/L) (median)	22.97±17.64 (17)	20.83±20.41 (14)	21.92±19.04 (16)	20.12
ALP (U/L)	71.41±21.65	74.46±23.13	72.9±22.39	10.29
Creatinine (mg/dL)	0.72±0.19	0.66±0.15	0.69±0.18	10.015*
Alb (g/dL)	4.51±0.45	4.5±0.36	4.51±0.41	10.91
Globulin (g/dL)	3.05±0.48	3.05±0.38	3.05±0.43	10.91
T. Cholesterol (mg/dL) (median)	175.53±33.22	174.32±42.07	174.94±37.74	10.8
Triglyceride (mg/dL) (median)	121.28±79.17 (101.5)	133.32±89.44 (114)	127.18±84.39 (106)	20.95

[1]Student's t-test, [2]Mann-Whitney U test, * p<0.05, **p<0.01
HgB: Hemoglobin B, HCT: Hematocrit, PLT: Platelet, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transpeptidase, ALP: Alkaline phosphatase

Table 4. Contuniued

	USG hepatosteatosis (grade)		p
	Mean ± SD	Median	
Patient (n=122)	0.52±0.72	0	0.004**
Control (n=117)	0.83±0.87	1	
Total (n=239)	0.67±0.81	0	

Mann-Whitney U test, **p<0.01, USG: Ultrasonography, SD: Standard deviation

hand, RF was found as 24.2% and ANA as 3% in CHB patients. LKM, SMA, and anti dsDNA were not observed in any of the both groups (8). Panasiuk (9), found that the rate of autoantibodies (ANA, AMA, ASMA) which were examined via the IFA method between the patients infected with hepatotropic viruses (HBV, HCV) and the patients with chronic liver disease was 23-28%. This rate

Table 5. Assessments regarding the ANA positivity in the control group and the ANA and ASMA in the patient group

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.07±8.44 (37)	42.5±10.14 (38.5)	[1]0.12
BMI (kg/m²)	26.94±4.85 (26.52)	28.43±4.51 (28.03)	[1]0.22
USG hepatosteatosis	0.54±0.74 (0)	0.42±0.51 (0)	[1]0.80
T.Cholesterol (mg/dL)	175.71±33.9 (172)	173.92±27.29 (171.5)	[1]0.93
Triglyceride (mg/dL)	120.31±81.06 (100)	130.17±61.18 (131.5)	[1]0.26
Gender n%			
Male	44 (40%)	6 (50%)	[2]0.54
Female	66 (60%)	6 (50%)	

[1]Mann-Whitney U test, [2]Fisher's exact test.
ANA: Antinuclear antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

Table 5. Contuniued

Control group	**ANA**		**p**
	Negative (n=107)	**Positive (n=10)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.42±10.37 (39)	35.8±10.18 (36.5)	[1]0.67
BMI (kg/m²)	27±4.79 (26.96)	28.25±8.11 (25.87)	[1]0.98
USG hepatosteatosis	0.79±0.85 (1)	1.3±1.06 (1)	[1]0.10
T. Cholesterol (mg/dL)	173.06±41.58 (172)	187.8±47.21 (193)	[1]0.38
Triglyceride (mg/dL)	137.79±91.6 (123)	85.5±38.78 (68.5)	[1]0.03*
Gender n%			
Male	44 (41.1%)	3 (30%)	[2]0.73
Female	63 (58.9%)	7 (70%)	

[1]Mann-Whitney U test, [2]Fisher's exact test, *p<0.05.
ANA: Antinuclear antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

Table 5. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
Age	37.4±8.59 (37)	41±10.92 (37)	[1]0.52
BMI (kg/m²)	27.08±4.85 (26.64)	27.25±4.53 (26.72)	[1]0.83
USG hepatosteatosis	0.55±0.73 (0)	0.14±0.38 (0)	[1]0.13
T. Cholesterol (mg/dL)	175.07±33.8 (172)	183.14±21.7 (169)	[1]0.43
Triglyceride (mg/dL)	122.48±80.94 (101)	101.57±38.01 (108)	[1]0.83
Gender n%			
Male	48 (41.7%)	2 (28.6%)	[2]0.69
Female	67 (58.3%)	5 (71.4%)	

[1]Mann-Whitney U test, [2]Fisher's exact test.
ASMA: Anti-smooth muscle antibody, BMI: Body mass index, SD: Standard deviation, USG: Ultrasonography

was found as 25% in the individuals with non-infectious chronic liver disease. In the study by Volchkova et al. (10), to examine the autoimmune parameters in acute viral hepatitis, SMA and ANA were diagnostically found at significant titers in the patients with acute viral hepatitis A, B and C. Codes et al. (11), investigated ANA and ASMA autoantibodies in the patients with acute viral hepatitis and found that autoantibodies could be found in acute viral hepatitis but they did not have any prognostic significance. In their study, Tage-Jensen et al. (12), observed that among the circulating autoantibodies such as LMA, SMA, ANA and AMA, ANA was dominant in the acute-phase serums of the patients with late progressing chronic liver disease. In Turkey, Afşar et al. (13), determined the frequency of ANA as 65.3%, AMA as 3%, SMA as 7% and LKM as 1% in 98 patients infected with CHC and found the frequency of ANA as 66.6% and SMA as 12.6% in 102 patients injected with CHB and the autoantibody frequency in the control group including healthy individuals as 14.4%. In their study, Bayram et al. (14), investigated ANA by using indirect fluorescent antibody (IFA) method in serum samples and HBsAg and anti-HBc immunoglobulin G (IgG) by using enzyme immunoassay method. As a result of the study, it was found that ANA was positive in 46 (54.7%) of a total of 84 patients. HBsAg and anti-HBc IgG were positive in a total of 20.2% (17/84) of the study group as 23.7% (9/38) of the patients with negative ANA and 17.4% (8/46) of the patients with positive ANA (14). The incidence rate of HBV in the patients included in the study was not different between the groups with positive and negative ANA and was higher in total compared to the normal population. It is considered that this result

Table 6. Assessment of HAI and fibrosis, HBeAg and Anti HBe positivity according to the ANA and ASMA positivity in the patient group

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
HAI	6.48±2.30 (6)	6.92±2.19 (6.5)	0.47
Fibrosis	1.76±1.11 (2)	2.17±1.19 (2)	0.25

Mann-Whitney U test.
ANA: Antinuclear antibody, HAI: Hepatic activity index, SD: Standard deviation

Table 6. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	Mean ± SD (median)	**Mean ± SD (median)**	
HAI	6.57±2.26 (6)	5.71±2.75 (6)	0.45
Fibrosis	1.81±1.11 (2)	1.71±1.25 (1)	0.87

Mann-Whitney U test.
ASMA: Anti-smooth muscle antibody, HAI: Hepatic activity index, SD: Standard deviation

Table 6. Contuniued

Patient group	**ANA**		**p**
	Negative (n=110)	**Positive (n=12)**	
	n (%)	**n (%)**	
HBeAg (IU/mL)	17 (15.5%)	1 (8.3%)	1.000
Anti-HBe (IU/mL)	92 (83.6%)	12 (100%)	0.210

Fisher's exact test.
ANA: Antinuclear antibody, HbeAg: Hepatitis B e antigen

Table 6. Contuniued

Patient group	**ASMA**		**p**
	Negative (n=115)	**Positive (n=7)**	
	n (%)	**n (%)**	
HBeAg (IU/mL)	18 (15.7%)	0 (0%)	0.592
Anti-HBe (IU/mL)	97 (84.3%)	7 (100%)	0.592

Fisher's exact test.
ASMA: Anti-smooth muscle antibody, HbeAg: Hepatitis B e antigen

may be related to the exposure of the patients in the selected group to frequent invasive procedures for chemotherapy and/or for diagnostic purposes.

In their study, Unal et al. (15), assessed the prevalence of immunomodulator and also cellular and humoral immune parameters and the prevalence of autoantibodies before the antiviral treatment in the patients with chronic HBV infection and they found ANA positivity as 18.2%. According to the data of this study, the formation of ANA is a part of the natural course of chronic HBV infection and may indicate the importance of clinical follow-up with the predisposition towards the autoimmune diseases. In their study, Michalska et al. (16), showed that the patients infected with HBV and HCV may explicitly show the clinical features of autoimmune diseases and thus attention should be paid for the selection of the required treatment. In the present study, AMA and anti-LKM1 were not seen in any case in the patient and control groups. ANA result in 9.8% of the cases in the patient group and in the 8.5% of the cases in the control group was positive and no statistically significant difference was found between them. ASMA results was positive in 5.7% of the cases in the patient group and in 0.9% of the cases in the control group and the difference between was found very close to significance but it was not found statistically significant. In all the studies reviewed, the prevalence of the autoantibodies was reported as higher than the present study. Interferon therapy conducted in some of the patients was considered as a triggering factor. In all the studies investigating the frequency of autoantibodies in CHB and CHC, it is observed that HBV, especially HCV, has induced the antibody formation. Advanced studies can enable to clarify the role of these antibodies in chroniczation and activation of the disease. The studies have revealed that antiviral drugs and the use of interferon increase the autoantibody formation. Thus, pre-treatment autoantibody screening in newly diagnosed patients with CHB and CHC may give insight into the progression of the disease and the possible autoimmune events and extrahepatic findings. Furthermore, in the present study, the hepatosteatosis level was significantly higher in the control group compared to the patient group. Hepatosteatosis is defined as the amount of fat in the liver, especially triglycerides, more than 5% of the liver weight or filling of more than 5% of hepatocytes by lipid vacuoles in the histopathologic examination. Obesity, alcohol, diabetes, hyperlipidaemia, infection, inflammatory bowel diseases, some drugs and chemical substances may lead to hepatosteatosis (17). There is no explicative reason in approximately 5% of hepatosteatosis. Previous studies showed the comorbidity of CHC and hepatosteatosis frequently and it is thought that hepatosteatosis is caused by the HCV (18). In their study, Vere et al. (19), showed that steatosis was more frequent in the patients with CHC than the patients with CHB. In the same study, although the sensitivity and specificity were lower compared to biopsy, it was revealed that ultrasonographically detected steatosis was histopathologically associated with fibrosis. In the study conducted by Altıparmak et al. (20), in the patients with CHB, it was found that mean age, BMI, cholesterol and triglyceride levels were higher in the steatosis group; no significant difference was found between the groups with and without steatosis in terms of AST, ALT, ALP, GGT, and viral load, and is was considered that steatosis was associated with obesity and hyperlipidaemia rather than the effect of the virus. The fact that hepatosteatosis was higher in the control group than patient group in the present study support that this is not associated with the effect of HBV. The prevalence of at least one of ANA, AMA and ASMA in the patients with hepatic steatosis was 23-36% (21). Although the positive ANA result with non-alcoholic steatohepatitis (NASH) was shown, the prevalence and importance of ANA positivity has not been clearly known. In a previous study, laboratory data of a total of 55 patients histologically diagnosed with NASH were retrospectively assessed. ANA was found to be positive in 14 (25%) of 55 patients. When comparing the groups with positive and negative ANA results, no statistical difference was found between the groups in terms of age, gender distribution, BMI, ALT, AST, GGT, ALP, albumin, total cholesterol, triglyceride and ferritin (22). In the patients with NASH, there is no sufficient number of studies investigating the prevalence of ANA. Also in the patients with NASH, the importance of ANA positivity is not known explicitly. Cotler et al. (23), found ANA positivity as 34% in 74 patients with NASH. They did not find any difference between the patients with NASH having positive and negative ANA results in terms of the laboratory parameters but they found that ANA positivity was higher in females. In the study by Loria et al. (24), ANA was found to be positive in 18 (21.4%) of 84 patients with NASH. While the patients having ANA positivity were older than the patients with negative ANA, no difference was found between two groups in terms of biochemical parameters. ANA is positive in approximately 71% of the patients with autoimmune hepatitis. However, such a positivity of the autoantibody does not mean that autoimmune liver disease is present (99). Since any component of the liver cells can trigger the formation of autoantibody, serum autoantibodies were found as positive in approximately 7-52 of the patients with chronic liver diseases due to various reasons (100-101).

Study Limitations

We conducted a retrospective study of the records. For this reason, there are some limitations. Our other limitation is that, we did not investigate the pathologist observation difference.

Conclusion

Consequently, all the studies investigating the frequency of autoantibodies in CHB have revealed that HBV induces the formation of antibody. Also, these studies reported the prevalence of the autoantibodies higher than the present study. Interferon therapy and antiviral drug therapy conducted in some of the patients is considered as a triggering factor. In addition, in the present study, the number of individuals with hepatosteatosis in the control group may have increased the frequency of the autoantibodies in this group. It is required to conduct numerous prospective studies following autoantibody positivity in newly diagnosed patients before and after treatment.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee Ministry of Health Bağcılar Training and Research Hospital (approval number: 2015/414).

Informed consent: Each of the patients included in the study signed an "informed consent form" that provides information about the study and states that the consent of the patient is taken.

Peer-review: Internally peer-reviewed.

Author Contributions

Concept: E.Y., Design: E.Y., U.A., Data Collection or Processing: F.E.P., U.A., M.G.A, M.Ö., A.B.E., Ü.S.T., Analysis or Interpretation: F.E.P., A.B.E., Ü.S.T., H.Y., Literature Search: E.Y., A.B.E, Writing: U.A., H.Y.

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