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A cross sectional study of autoantibodies in children with hepatitis A infection

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Abstract

Background: *Hepatitis A is one of the most frequently reported vaccine-preventable diseases worldwide and remains endemic in many areas of the world. The study aims to investigate the prevalence of autoantibodies in patients with acute viral hepatitis A.*

Method: *A total of 52 patients diagnosed with hepatitis A (HA) were enrolled in this study. The diagnosis of acute hepatitis A (AHA) was based on negative hepatitis B surface antigen and hepatitis C virus RNA, and positive immunoglobulin (Ig) M HA antibody. Biochemical parameters and the presence of autoantibodies were recorded.*

Results: *Prolonged protrombin time, international normalized ratio corrected with vitamin K and prolonged activated partial thromboplastin time were seen in 11, 11 and in 12 patients respectively. Total bilirubin levels were higher than 2 mg/dL in 21 patients and only five of these patients had direct bilirubin higher than 50% of the total bilirubin and serum bilirubin levels were higher than 10 mg/dL during at least 4 weeks. These 5 patients were evaluated as cholestatic hepatitis. Antinuclear antibodies were detected in four and anti-liver cytosolic antigen type 1 in one patient.*

Conclusion: *The early diagnose of any autoimmune disease in patients with HA was found important since it may help take necessary precautions. In addition, we conclude that further studies in larger populations will contribute to understanding of the relationship between the autoimmune disease and HA.*

Keywords: Seroprevalence, autoantibodies, hepatitis A, infection

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Introduction

Hepatitis A (HA) is one of the most frequently reported vaccine-preventable diseases worldwide and remains endemic in many areas of the world.[1] HA, although with a mild course during childhood, may cause significant morbidity and mortality among both adolescents and adults.[2] In addition, acute hepatitis A (AHA) has been known to trigger autoimmune diseases. Nevertheless, few cases of autoimmune hepatitis (AIH) following AHA have been described.[3,4]

One of activities of the immune system is to protect the body from viruses, bacteria, and other living organisms. The immune system usually does not react against the body's own cells. However, sometimes it attacks the cells it is supposed to protect, which is known as autoimmunity. It is thought that certain bacteria, viruses, toxins, and drugs trigger an autoimmune response in people who are genetically susceptible to developing an autoimmune disorder. Hence, many reports have detailed the frequency and clinical impact of serum autoantibodies in HCV (hepatitis C virus) and HBV (hepatitis B virus),[5-12] though their association with clinical features remains controversial.[5,6,8,9] However, there are few reported cases of autoimmune hepatitis after HAV infection.[13-17] Despite evidence that inherited factors may play a role in the development of autoimmunity after viral infection, the pathomechanism remains unclear.

Autoimmune disorders encompass a wide spectrum of diseases that progress with several clinical findings. They can be organ-specific such as AIH, Hashimoto's thyroiditis, or they can involve multiple organs such as systemic lupus erythematosus (SLE). The common characteristic of all these disorders is the production of different autoantibodies against various autoantigens along with inflammation. For instance, AIH is associated with antinuclear antibodies (ANA), antismooth muscle antibodies (ASMA), antimitochondrial antibodies (AMA), anti-liver/kidney microsomal antibody (ALKM1) and anti-liver cytosolic antigen type 1 (ALC-1) while Hashimoto's thyroiditis is associated with tyroid peroxidase (TPO), celiac disease is associated with antigliadin antibodies (AGA), antiendomysial antibodies (EMA), and systemic lupus is associated with double-stranded deoxyribonucleic acid (ds-DNA). Rheumatoid factor testing can also help diagnose autoimmune conditions, including rheumatoid arthritis (RA), Sjogren's syndrome, and SLE.

Although there are many literatures3-10 indicating the evidence that particularly HCV and HBV are among the hepatotropic viruses stimulating autoimmune reactions, little exists how frequent and at which stage hepatitis A stimulates these reactions. The aim of this study is to investigate the prevalence of autoantibodies in early stage in patients with acute viral hepatitis A.

Materials and Methods

The parents of all subjects gave informed consent prior to the study entry and the study was conducted in accordance with the Declaration of Helsinki. A total of 52 patients diagnosed with hepatitis A, admitted to the Department of Pediatrics, University of Dicle (Diyarbakır, Turkey) were enrolled in this prospective study. They were diagnosed with acute hepatitis A (AHA) based on negative hepatitis B surface antigen and hepatitis C virus RNA and positive immunoglobulin (Ig) M HA antibody. The study included only uncomplicated AHA patients. Therefore two patients diagnosed with fulminant hepatitis as a result of AHA were excluded from the study Characteristics (age and sex) and the following laboratory findings at presentation in the blood sample of each patient were recorded: Immunoglobulin (Ig) M HA antibody, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), albumin, globulins, total bilirubin (TB), indirect bilirubin (IB), direct bilirubin (DB), platelet count (PLT), hemoglobin (Hb), white blood cell (WBC), prothrombin time rate (PTT), international

normalized ratio (INR), activated partial thromboplastin time (aPTT), ANA, ASMA, AMA, ALKM1, ALC-1, AGA (IgA, IgG), EMA (IgA, IgG), antibodies to ds-DNA titers, TPO antibodies titer and RF. AST, ALT, ALP, GGT, albumin, globulins, TB, IB and DB were measured from centrifuged venous blood by enzymatic colorimetric method performed with Abbot ARCHITECT 1600. Hemoglobin (Hb) level, WBC and PLT were measured by hemocounter (Cell-Dyne 3700, Abbott, USA, 2005 and 2008). Prothrombin time rate, INR and aPTT were measured by coagulometry (Sysmex CA 7000, 2010). Immunoglobulin (Ig) M HA antibody was determined by electrochemiluminescence immunoassay kits which are commercially available (Roche Diagnostics, Mannheim, Germany, Cobas 6000), Anti-HCV and hepatitisits B surface antigen (HBsAg) were determined by ELISA method with Cobas 6000 instrument (Roche). Rheumatoid factor (RF) was measured by nephelometric method (N Latex RF kit II; Dade-Behring BN II, Marburg, Germany). The values equal or higher than 15 IU/mL were accepted as elevated or positive. ANA, AMA, ASMA, ds-DNA, AGA-A, AGA-G were manually measured by indirect immunofluorescence assay (IFA) method. Type 1 anti-liver/kidney microsomal antibody (ALKM1) and anti-liver cytosolic antigen type 1 (ALC-1), were carried out in all patients using the blood technique. The chemiluminescence assays for detection of autoantibodies to thyroid peroxidase (TPO) on the IMMULITE 2000 system ((Diagnostic Products Corporation (DPC), Los Angeles, USA) were evaluated.

To our knowledge, the earliest time of the development of autoimmune reaction observed in patients with AHA was seen as 7th week.18 Therefore, the patients were divided into two groups based on the time they spent from the complaint to diagnosis to see the effect of timing on the development of autoimmune reactions. The first group is formed from patients who had spent longer time (spending more than 4 weeks after complains) in different medical centers before applying to our clinic while the other is formed from those who directly applied to our clinic (spending less than 4 weeks). The first group involves 17 (32.7%) patients (9 girls and 8 boys) while the second group involves 35 (61.3%) patients (17 girls and 18 boys).

Statistical analysis

Statistical analyses were performed using the SPSS 18.0 software package programme. Normal distributed continuous variables were presented as mean ± standard deviation (SD) and compared by an unpaired Student′s t test. Categorical variables were presented as frequencies (%) and compared using a Pearson chi-square. Statistical significance was defined as $P < 0.05$.

Result

The demographic and laboratory characteristics of patients in this study are summarized in Table 1. No significant difference in parameters studied was found in terms of age and sex.

Higher values of WBC were found in 7 (13.5%) whereas Hb and PLT were detected in normal range in all of patients. Serum aminotransferases, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were markedly elevated in all patients, with a greater increase in ALT in the most cases. Alkaline phosphatase levels were higher in 7 (13.3 %) patients and GGT levels were higher in 22 (10.6%) patients while serum albumin levels were lover in 8 (15.4 %) patients and serum globulin levels were higher in 15 (28.8 %) patients. Prolonged protrombin time and INR corrected with vitamin K and prolonged aPTT were seen in 11 (20.9 %), 11 (20.9 %) and in 12 (23%) patients respectively. TB levels were higher than 2 mg/dL in 21 (%40.4) patients and only five of these patients had direct bilirubin higher than 50% of the total bilirubin and serum bilirubin levels were higher than 10 mg/dL during at least 4 weeks. These five patients were

evaluated as cholestatic hepatitis. Antinuclear antibodies were detected in 4 (7.6 %) patients with cholestatic hepatitis. Other autoantibodies were negative in all patients.

Table 1. Demographic and laboratory characteristics of patients with acute hepatitisis A in this study.

Parameters	normal range	mean±SD	range
Age (years)		7.69±2.85	3-14
Sex (F/M)		26/26	
HAV-IgM COI	<1	10.14±3.94	1.60-16.92
ALT (U/L)	5-45	1116±713	35-4113
AST (U/L)	5-55	940±773	23-4202
GGT (U/L)	5-64	104.2±125	7-493
ALPU/L	145-525	339±141	151-727
Albumin (g/dL)	3,5-5	3.6±0.43	2.1-4.5
Globulin (g/dL)	2.6-3.7	3.6±0.41	2.7-4.7
TB (mg/dL)	0.2-1.2	5.22±4.79	0.3-25.0
IB (mg/dL)	0.2-0.7	1.90±3.49	0.1-24.0
Hg g/dL	12-16	12.07±0.36	11.33-12.7
WBC (1000/UL)	4.60-10.20	8.83±2.39	4.1-19
Platelet (1000/UL)	142-424	258±81	107-453
INR	0.88-1.2	1.30±0.39	0.95-3.50
aPTT(sec)	25-35	33.04±5.36	23.5-47.1
PTT (sec)	10-14.5	15.88±5.32	11.0-44.2
RF (IU/ml)	0-15	10.65±0.98	9-14
ANA(titer)	<1/40	positive in 6	1/40-1/80

HAV-IgM: hepatit A virus-Immunoglobin M, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase GGT: γ-glutamyl transpeptidase, TB: total bilirubin, IB: indirect bilirubin, DB: direct bilirubin, WBC: white blood cell, PTT: prothrombin time rate, INR: international normalized ratio, aPTT: activated partial thromboplastin time, RF: rheumatoid factor, ANA: antinuclear antibodies.

It was shown that none of the patients in the second group had positive autoantibody while 6 in the first group had positive ANA although it was not regarded as a diagnostic evaluation; three with 1/40, three with 1/80 titers and one had ALC-1 positive. Interestingly, these patients had significantly lower HAV-IgM HA titers compared with the rest of the patients. In the other word, it was found that HAV-IgM HA levels were significantly lower (5.99±3.39) in the first group compared to that (12.17±2.25) in the second group ($p<0.001$). However, significant difference was not found in other parameters when two groups are compared.

Correlation analyses between parameters studied are listed in Table 2. It shows that the mean age is note correlated with any parameters. In addition, HAV (Ig) M HA titers are only negatively correlated with globulin. Alanine amino transferase was positively correlated with AST and GGT while it was negatively correlated with PLT. Aspartate amino transferase was positively correlated with PT and INR while it was negatively correlated with PLT. γ-Glutamyl transpeptidase was positively correlated with ALP while it was negatively correlated with globulin. Albumin was negatively correlated with globulin, PT, INR and TB while globulin was positively correlated with PT, INR. TB was also positively correlated with PLT, PT and INR.

Discussion

Hepatitis A is a common cause of viral hepatitis in humans and is usually a self-limiting disease that resolves within a few weeks after onset.[19] HAV infection is probably one of the several triggers that may induce autoimmune liver diseases. Autoimmune liver diseases can be divided into the cholestatic form, namely primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)

and the hepatic form, autoimmune hepatitis (AIH). Autoimmune cholangitis (AIC) is a newly identified disease entity that includes patients with all the clinical, biochemical and histological characteristics of PBC, but lacking a positive serum antimitochondrial antibody (AMA) and frequently have a high titre serum ANA.[20] In our study, cholestatic hepatitis was found in five patients and four of these patients had ANA positive. According to the literature data although the exact cause of cholestatic hepatitis is not well-known, the effectiveness of immunosuppressive therapy such as corticosteroids, azathioprine makes one to think that autoimmunity plays role in physiopathology.[21,22] The positive tendency in ANA titre levels in 4 cholestatic hepatitis patients in our study also suggests that autoimmunity may be involved in the physiopathology of the disease. The reason why ANA titres were not so high in these patients may be ascribed to the fact that these titres are recorded during the acute period of the patients.

Table 2. Pearson's correlation analysis between parameters.

Pairs		**r**	**p**
HAV-IgM	Globulin	-0.357	0.009
ALT	AST	0.884	<0.001
	GGT	0.370	0.007
	PLT	-0.386	0.005
AST	ALT	-0.321	0.02
	PTT	0.390	0.04
	INR	0,481	<0.001
ALP	GGT	0.447	<0.001
Albumin	Globulin	-0,296	0,033
	PTT	-0.571	<0.001
	INR	-0.571	<0.001
	TB	-0.432	0.001
Globulin	PTT	0.358	0.009
	INR	0.290	0.037
	GGT	-0.308	0.027
	Platelet	0.266	0.057
TB	PTT	0.480	<0.001
	INR	0.477	<0.001
PTT	INR	0.983	<0.001
	aPTT	0.425	0.002
INR	aPTT	0.346	0.013

Nevertheless the development of autoimmune hepatitis (AIH) after HAV infection albeit scarce has been reported.[23-28] Such reports have described that HAV infection seemed to trigger the development of autoimmune reaction. AIH is a rare disease with unknown etiology, causing chronic necroinflammatory changes in the liver. The exact molecular, cellular, pathologic and physiological mechanisms leading to autoimmunity are not yet fully explained. Infectious agents like hepatitis viruses, on the other hand, have often been named as triggers of autoimmune diseases. Hepatitis C virus has existed with AIH more than other hepatitis viruses.[8] Viral proteins of the viruses may be similar to the amino acid chain of different autoantigens in the liver. Therefore, cross immune reactions to viral proteins may also cause the damage in liver tissue at the same time.[9,10]

However, to our knowledge there are not many studies investigating the immune reactions during the early stages of acute hepatitis, and excessive immune reactions during acute HA have not been documented. While immune reactions are not often assessed in acute HA, as it was reported in one patient with AIH[29], excessive immune reactions may occur during acute HA. One patient was also reported with a deficiency of specific suppressor T cells for asialoglycoprotein receptor who developed type I AIH following subclinical HA, suggesting the possibility that immunological

abnormalities including antigen presentations were involved in the onset of AIH following acute HA.[17] Based on the serologic heterogeneity of autoantibodies, at least three subgroups of autoimmune hepatitis have been described.[30] Antinuclear antibodies (ANAs) characterize classical autoimmune-type lupoid hepatitis (AIH-1).[31] In type 2 AIH, anti-LKM1 autoantibodies are associated with the presence of anti-liver–cytosol type 1 (anti-LC1) autoantibodies in 30% of patients. In 10% of type 2 AIH, anti-LC1 are the only liver-related autoantibodies in the circulation.[32] These autoantibodies are not pathogenic but anti-LC1 concentration in AIH patient sera are correlated with transaminase levels, and, therefore, with liver inflammatory activity.[33] In our study, ALC-1 was positive only in one patient, who was not one of those having cholestatic form of hepatitis and other autoimmune antibodies was not positive in this patient. A third subgroup, type 3 (AIH-3), is associated with antibodies against a soluble liver antigen.[34] This type was not observed in any of the patients.

Conclusion

The investigation of patients undergoing acute hepatitis A in terms of autoimmune reaction and a detailed follow-up is important since it is possible to diagnose any autoimmune disease in an earlier stage even in the acute period of infection and thus this might help take necessary precautions. In addition, we suggest that further studies in larger populations will contribute to a comprehensive understanding of the relationship between the autoimmune disease and acute hepatitis A.

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