Acute hepatitis B due to immune-escape mutations in a naturally immune patient

Sabahattin Kaymakoglu[1], Bulent Baran[1], Derya Onel[2], Selim Badur[2], Tanju Atamer[3], Filiz Akyuz[1]

(1) Department of Gastroenterohepatology, (2) Microbiology, (3) Hematology, Istanbul University, Istanbul Faculty of Medicine, Capa, 34093, Istanbul, Turkey.

Abstract

Incidence of hepatitis B virus infection (HBV) has been greatly reduced globally after the introduction of universal vaccination programs. However, another potential threat was noticed almost 2 decades ago, which is the selection of antibody escape HBV strains. Antibody or immune escape strains of HBV carry mutations in the S gene which encodes "a" determinant region located at amino acid positions 124 to 149. Certain mutations in this region, which promotes antibody response, might lead to an alteration in the antigenicity of hepatitis B surface antigen (HBsAg). Anti-HBs might fail to neutralize the mutant virus and transmission or reappearance of infection in previously immunized individuals can be possible. Herein, we report a patient with known HBV seropositivity (HBsAg negative, anti-HBs positive, anti-HBc IgG positive) for more than 10 years who developed a symptomatic acute hepatitis due to occurrence of immune escape mutants in the absence of any immunosuppression or cytotoxic chemotherapy. To the best of our knowledge, this is the first reported case of acute hepatitis B due to escape mutations in a naturally immune patient. (Acta gastroenterol. belg., **2014**, 77, **262-265**).

Key words : S gene, mutation, acute hepatitis B, immune-escape.

Introduction

During the last 20 years, primary prevention by vaccination against hepatitis B virus (HBV) has been the main driving force to control incidence of chronic infection worldwide. After the introduction of universal vaccination programs throughout the world, the incidence of acute and chronic hepatitis B infection was significantly decreased (1,2). It has been recognized more than two decades ago that the administration of HBV vaccine can result in the selection of antibody escape variants especially in countries with a high prevalence of HBV infection (3). In high-prevalence countries such as China, Thailand and Taiwan occurrence of immune-escape mutants however did not alter the success of the vaccine program and the overall burden of hepatitis B was reduced (4,5). Immune escape strains of HBV carry mutations in the S gene which encodes "a" determinant region located at amino acid positions 124 to 149 (6). The major B-cell neutralizing epitopes of hepatitis B surface antigen (HBsAg) reside in this region which promotes antibody response. Certain mutations in the "a" determinant might lead to an alteration in the antigenicity of HBsAg protein, and so antibody against HBsAg (anti-HBs) might fail to neutralize the virus (7). Transmission of infection to immunized individuals with immune-escape mutants is therefore possible. In addition, occurrence of mutant strains in a naturally immune patient can be encountered especially in patients receiving chemotherapy or long-term immunosuppression. However, development of symptomatic hepatitis has not been reported in an immunocompetent patient with natural immunity against HBV. Herein, we report a naturally immune patient who presented with symptomatic acute hepatitis B after emergence of S gene mutations in the absence of previous immunosuppression.

Case report

A 71-year-old male patient with a history of left-sided ulcerative colitis for 4 years was referred to our centre with an acute hepatitis, while he was under clinical evaluation in another hospital for mild anaemia (haemoglobin 10.8 g/dl), high erythrocyte sedimentation rate (73 mm/hour and C-reactive protein (110 mg/dl) with a possible diagnosis of chronic myelomonocytic leukaemia. His past medical history was also remarkable for a coronary by-pass operation 2 years ago and treated pulmonary tuberculosis during childhood. The patient was in clinical remission under 5-aminosalicylate therapy (mesalamine 4 g/day) and he did not report any relapses requiring corticosteroid therapy. He was initially evaluated to determine aetiology of acute hepatitis in the former hospital and laboratory investigations at that time showed increased alanine and aspartate aminotransferase levels (ALT : 936 IU/l, AST : 762 IU/l) with direct hyperbilirubinemia (total bilirubin 4.3 mg/dl) and mildly elevated alkaline phosphatase (206 IU/l, normal range : 15-185 IU/l)) and gamma-glutamyl transferase levels (68 IU/l, normal range : 8-60 IU/l). Serum albumin was normal (4.04 g/dl) and serum gamma globulin level (2.07 g/dl) was slightly elevated. Serological tests for HBV from 2009 were available (HBsAg negative and anti-HBs > 1000 U/ml) and his doctors did not repeat HBV serology at the first instance. Antibody to hepatitis C virus, hepatitis C virus RNA and immunoglobulin M antibody to hepatitis A virus were negative ; and antinuclear antibody (ANA) was positive (1/320 titre). Due

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Correspondence to : Prof. Sabahattin Kaymakoglu, M.D., Istanbul University, Istanbul Faculty of Medicine, Department of Gastroenterohepatology, Capa, 34390, Istanbul, Turkey. E-mail : kaymakoglus@hotmail.com

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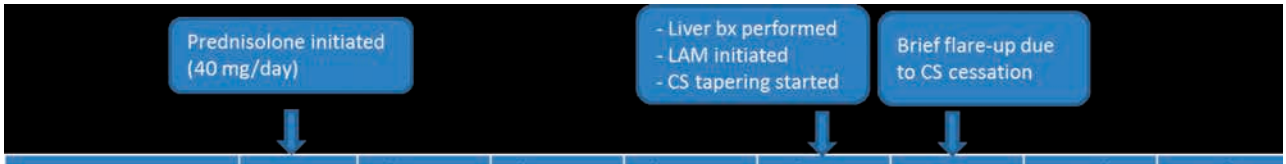

Laboratory parameters	Baseline	2nd week	4th week	8th week	12th week	LAM 1st mo	LAM 3rd mo	LAM 6th mo
AST (IU/l)	762	172		33	42	119	27	52
ALT (IU/l)	936	476		52	74	225	52	32
ALP (IU/l)	206	NA		60	58	64	37	24
GGT (IU/l)	68	NA		48	32	68	31	
Total bilirubin (mg/dl)	4.3	3.9		0.7	0.7	0.9	0.6	
HBsAg	Negative*	Positive			Positive		Positive	Negative
Anti-HBs (U/ml)	>1000*	>1000			>1000		>1000	>1000
HBeAg	NA				Positive		Positive	Negative
Anti-HBe	NA				Negative		Negative	Positive
HBV-DNA (IU/ml)	NA				16100		51	<20

Fig. 1. — Summary of the follow-up of the patient.
*Test results available in 2009 ; AST = Aspartate aminotransferase ; ALT = Alanine aminotransferase ; ALP = Alkaline phosphatase ; GGT = Gamma-glutamyl transferase ; NA = Not available ; bx = Biopsy ; LAM = Lamivudine ; CS = Corticosteroid ; mo = Month.

to the combination of negative virological markers, ANA positivity and mild hypergammaglobulinemia, his doctors decided to initiate prednisolone 40 mg/day with a possible diagnosis of autoimmune hepatitis. Transaminase levels were gradually decreased under corticosteroid therapy, but did not normalized and fluctuated between 1-2 times of upper limit of normal (Fig. 1). At 2nd week of the corticosteroid therapy, repeated virological markers revealed a positive HBsAg and anti-HBs (> 1000 U/ml). The patient was referred to our centre at 12th week of corticosteroid therapy and a detailed virological panel resulted HBeAg positive, anti-HBe negative, HBV-DNA 16.100 IU/ml. Antibody against hepatitis D virus was negative. We re-evaluated the medical history and previous laboratory records of the patient. He had no transfusion history and had not received any active or passive immunization against HBV infection. The patient had been tested for HBsAg, anti-HBc total and anti-HBs in 2000 and 2009. HBeAg/anti-HBe serology was also available in 2009. The results of HBV serology had shown a negative HBsAg, positive anti-HBc and anti-HBs in both occasions. HBeAg was negative and anti-HBe was positive in 2009. This information made us to think about the actual mechanism behind this clinical scenario, which might be reactivation of hepatitis B virus after occurrence of immune-escape mutations or a re-infection with a mutant strain. A mutation analysis of S gene region was performed by using the amplification primers and Sequence Reagent Mix-DYEnamic ET Terminator Cycle Sequencing Kit (*Amersham Pharmacia, The Netherlands)*) on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA), and 3 point mutations including T125M, P127T, D144V were detected. A liver biopsy was carried out to determine the severity of the liver disease and histological examination showed fibrous expansion of most portal areas with short fibrous septa (Ishak stage 2) and histological activity index was 7. Ground-glass hepatocytes were observed in the histological examination. Lamivudine 100 mg/day was initiated ; ALT normalization and undetectable HBV-DNA (< 20 IU/ml), HBeAg conversion and HBsAg loss was achieved at 6th month of the treatment (Fig. 1). At the last follow-up visit (9th month of lamivudine treatment) the patient had no symptoms, liver function tests and ultrasonography were normal, virological test results were as follows : HBsAg negative, HBeAg negative, anti-HBe positive and HBV-DNA < 20 IU/ml. Lamivudine was withdrawn at 9th month and currently the patient is doing well under follow-up without medication.

Discussion

In the present case, we report an elderly male patient who was known to be naturally immune against HBV infection for more than 10 years. He developed a symptomatic acute hepatitis due to immune-escape HBV infection with S gene mutations in 3 points ; T125M, P127T, D144V. Although an early diagnosis was not available, biochemical remission and undetectable HBV-DNA and HBsAg was achieved by lamivudine monotherapy in 6 months.

HBV with S mutations has been reported in a variety of patient groups ; however the clinical significance of this mutation is still unclear. The great majority of patients who carry S mutant strains are children infected with vaccine-escape mutants after administration of

widespread immunoprophylaxis in high-prevalence countries (4,5). Immune-escape mutant strains may emerge by also 3 other mechanisms : selection of mutant strains after immunoprophylaxis failure in orthotopic liver transplant recipients (8), development of S gene mutations in patients receiving nucleos(t)ide analogues (9) and during low-level viral persistence either in blood or liver of HBsAg-negative patients which is the phenomenon widely known as occult hepatitis B (OHB) (10). OHB is generally accepted as a benign clinical condition, except having an impact on blood transfusion, organ transplantation, immunosuppression, and HCC development (11). It is known that the patients with serological evidence of previous HBV infection may develop clinical hepatitis under immunosuppression or chemotherapy, especially after rituximab treatment, and activation under immunosuppression occurs almost always in patients with a positive antibody against HBV core antigen (12,13). In patients with previous exposure to HBV, immunosuppressive therapy may lead to decline of anti–HBs titres, and in several patients this might be accompanied by reappearance of HBsAg and clinical hepatitis (12). Uncommonly, some patients with OHB under immunosuppression may demonstrate increased HBV-DNA without reappearance of HBsAg which is definitely associated with immune-escape mutations (14-16).

It is a known issue that S gene mutations may lead to false-negative results for diagnosis of HBV infection. A diagnostic assay used for screening may not show immunoreactivity if the assay configuration cannot detect mutants in the "a" determinant region (17). Anti-HBs can be positive which may provoke a wrong impression for the replication status of HBV. In fact, anti-HBs cannot neutralize HBsAg with certain mutations and clinical hepatitis may occur regardless of anti-HBs levels. Later generation HBsAg assays have improved reagent configurations that allow them to detect HBsAg mutants (18). In our case, we used micro-particle enzyme immunoassay 5.0 (*Dade Behring, Germany)* to detect mutant HBsAg in the serum which provided an accurate diagnosis for the aetiology of acute hepatitis.

In the present case, one possibility is that the patient might have acquired the infection from another person infected by mutant HBV. Currently there is evidence that these mutations are stable and can be transmitted horizontally or vertically (19). In this scenario, the patient should have been in contact to a person with selective suppression of wild-type HBV ; including an immunoprophylaxis history (a liver transplant recipient or person who was born to an HBsAg-positive mother) or nucleos(t)ide analogue therapy. In our patient, a suspicious contact was not documented and this scenario is highly unlikely. We can assume that our patient had OHB for many years before developing a clinically overt acute hepatitis, but we do not have any past HBV-DNA results to support this. However, indirect evidences such as the presence of fibrosis in liver biopsy and known seropositivity for resolved HBV since 2000 strongly suggest presence of OHB. Besides, it is unique for a patient with OHB to develop an acute hepatitis in the absence of any immunosuppressive therapy. The patient was under follow-up by a specialist during the previous year for a possible diagnosis of chronic myelomonocytic leukaemia, so we were definitely sure that he did not receive transfusion or any immunosuppressive medication before acute hepatitis. Also our patient was not exposed to any external selective pressure in favour of S gene mutants. Although there is evidence demonstrating that S gene mutations can occur naturally in OHB (10), to the best of our knowledge a case of acute hepatitis due to immune-escape mutations in an OHB setting has never been reported before. Our experience in this case suggests that immune-escape mutations in OHB might be clinically more important than has ever been thought, and use of later generation HBsAg assays is mandatory to establish a timely and accurate diagnosis. Anti-HBs cannot neutralize the mutant virus in these patients, whether it is acquired by vaccination or previous resolved infection. OHB may pose risks not only in patients undergoing immunosuppression or receiving transfusion, but also in previously healthy patients.

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