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Case report

A chronic hepatitis B patient infected with HBsAg diagnostic-escape strain in the presence of anti-HBs positivity

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Abstract

Hepatitis B virus (HBV) is an important pathogen responsible for serious diseases like chronic hepatitis, cirrhosis and liver cancer. Turkey is located in the intermediate endemic region based on the average HBV surface antigen positivity. The high replication capacity of HBV and the lack of proofreading activity of the reverse transcriptase (RT) enzyme makes the virus prone to mutations. In this study, S gene mutation which is detected in a chronic HBV case without any follow-up and treatment is presented. Although patient was HBsAg negative and anti-HBs positive in the examinations, the mutation analysis was performed upon the high level of HBV DNA load and sG145R (vaccine escape) mutation in S gene region and rtI169F, rtV173L, rtA181G and rtT184R mutations in pol gene region were detected. S gene region mutations may cause false negatives in diagnostic assays that detect HBsAg. At the same time, it is a public health problem because these HBV variants can also be transmitted by vertical and horizontal routes. Therefore, when atypical serological profiles are encountered, it is of utmost importance to remember S gene mutations and perform necessary analyses.

Keywords: *HBsAg diagnostic test, vaccine-escape HBV variant, false negative HBsAg*

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Introduction

Hepatitis B virus (HBV) is an important pathogen responsible for serious diseases like chronic hepatitis, cirrhosis and liver cancer (1). Worldwide, more than 350 million people are chronic hepatitis B (CHB) carriers and about 800 thousand people die each year due to HBV-related complications (2, 3). In Turkey, HBV surface antigen (HBsAg) positivity is around 4%, and our country is in the intermediate endemic region (4).

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In the life cycle of HBV, pregenomic RNA is converted to DNA through reverse transcription. The number of mature virions released after completing all the steps in hepatocytes is 10^{11}-10^{13}/day. This high replication capacity and the lack of proofreading activity of the reverse transcriptase (RT) enzyme makes the virus prone to mutations (5).

The genome encodes four overlapping open reading frames (ORFs), C, S, P and X which are respectively translated into viral core protein, surface proteins, polymerase/RT and HBx. Mutations in the S gene region may lead to occult HBV infections, HBV reactivations and failures in the detection of HBsAg in diagnostic tests (6). In this study, we present a case of non-treated CHB with false HBsAg negativity and vaccine escape mutation in the S gene region as detected by molecular analysis.

Case report

A 57-year-old male patient was admitted with shortness of breath and abdominal pain in January 2019. Ultrasonographic (USG) examination of the abdomen revealed multiple solid lesions with a maximal size of 6.5 cm in the right liver lobe and consistent findings in portal vein with thrombus. Laboratory results after admission to hospital for further investigations were; *alanine aminotransferase (ALT)*: 30 U/L (normal range; NR, 0-50), *aspartate aminotransferase (AST):* 69 U/L (NR, <50), gamma-*glutamyltransferase (GGT):* 161 U/L (NR, 0-55), alkaline phosphatase (ALP): 264 U/L (NR, 30-120), total bilirubin: 1.23 mg/dl (NR, 0.3-1.2), prothrombin time (PT): 15.4 seconds (NR, 8.7-12.8), international normalized ratio (INR): 1.46 (NR, 0.8-1.2) and *alpha-fetoprotein (AFP):* 128.64 µg/L (NR, 0-9). The complete blood count was unremarkable except for an elevated white blood cell count (15.890/µL). His serology was found to be negative for HBsAg and negative for antibodies to hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Further investigations revealed that the patient was diagnosed with HBV infection 6 years ago, but he neither went to follow-up visits nor did he receive any treatment. At that time, HBsAg was positive, anti-HBs was negative and HBV DNA was 57844 IU/mL (Table-1). In the liver biopsy, *histological activity index* (HAI) and fibrosis scores were 14/18 and 4/6, respectively. Histological evaluation was performed according to modified Ishak scoring yielding results in correlation with CHB infection. Anti-HBc IgM positivity at low titers can be seen in the course of chronic active HBV infections (7). The patient's serological markers were retested, and additional examinations were requested.

Contrast-enhanced magnetic resonance imaging (MRI) of the abdomen demonstrated a solid

Table 1. Past and current serological test results of the patient

Test	Reference range	Unit	2013	2019
HBsAg	<0.9	COI	Positive	Negative/negative[a]
Anti-HBs	<10	IU/L	Negative (8.82)	Positive (197.3)
HBeAg	<1.0	INDEX	Negative	Negative
Anti-HBe	>1.0	COI	Positive	Positive
Anti-HBcIgM	<1.0	COI	Positive (1.83)	Negative
Anti-HBc (IgM+IgG)	>1.0	COI	Positive	Positive
Anti-HDV Total	Negative		Negative	NA[b]
HBV DNA[c]	Negative	IU/mL	57844	45847

[a] Test was performed twice with ElecsysHBsAg II; [b] NA, not available; [c] Quantitative HBV DNA PCR test, performed on Rotor Gene Q instrument

mass lesion (17.5x14x17 cm) in the right liver lobe suggestive of *hepatocellular carcinoma* (HCC). Several lesions of smaller size with similar characteristics were detected in the lesion-adjacent regions and left lobe. In serological analysis, HBsAg test was negative for the second time with the same test kit (Elecsys HBsAg II, Roche) while HBeAg was negative, anti-HBe was positive, anti-HBs was positive (197.3 IU/L), anti-HBc IgM was negative, anti-HBc (IgM+IgG) was positive and HBV DNA was 45847 IU/mL (quantitative HBV DNA PCR test, studied with Rotor Gene Q device) (Table-1). HBV *pol* / S gene mutation analysis was performed by Sanger di-deoxy sequencing method. The Geno2pheno drug resistance tool was used to this end. S [HBsAg protein; amino acids (aa) between 140 and 227] and *pol* gene (RT region; aa80-291) regions were examined. The sample was identified as genotype D, subgenotype D2 and sG145R mutation (vaccine escape mutation) was detected in S gene location (Table-2).

During the follow-up period, his clinical condition deteriorated; he was intubated, transferred to the intensive care unit and died within the same day.

Discussion

”a” determinant, between the aa residues 124 and 147, of HBsAg is normally a highly conserved region and responsible for the neutralizing antibody response to the virus (8). A mutation in this region causes significant changes in the three-dimensional structure of the protein. Structurally modified protein can escape vaccine-induced immunity and may cause false negative results in diagnostic assays that detect HBsAg (9). In the HBsAg protein, the transformation of glycine at position 145 to arginine (sG145R) is one of the major escape mutations and its frequency has increased in recent years (10). Escape mutants in this region may occur as a result of selective pressure of vaccine and / or HBV immunoglobulin (HBIg) administrations and antiviral therapies (nucleos(t)ide analogues), as well as in the natural course of HBV infection (6, 9). Some mutations in the S gene may also lead to changes in the *pol* gene region due to partial overlap between these genes in the genome sequence and they may cause compensatory mutations (11, 12).

The majority of the test kits target the “a” determinant region of the HBsAg protein. Therefore, some escape mutants may not be detected by commercially available assays (13). The performance of the tests in the market in detecting natural and mutant HBsAg particles were evaluated, and generally high sensitivities in correlation with one another were reported . Some kits could detect all strains successfully, while certain HBV variants were undetectable with more than one kit. Within this context, many studies to evaluate the performance of Elecsys HBsAg II have also been conducted. Most of these studies have shown that the assay is successful to detect all mutant strains and these HBV variants have been demonstrated to have no effect on the perfor-

Table 2. Mutations in HBV S and *pol* gene regions and their interpretations

HBV Genome Region	*Mutation pattern*	Comment
S gene	sG145R	Escape from vaccine, HBIg and diagnostic tests (high efficacy)
pol gene	rtV173L, rtA181G	Lamivudin; *compensatory mutations*
	rtA181G	Adefovir; aa change whose effect is unknown
	rtI169F, rtT184R	Entekavir; aa change whose effect is unknown
	-	Tenofovir; *susceptible*
	rtA181G	Telbivudin; aa change whose effect is unknown

mance of the assay (14, 15). On the other hand, in another study by Thibault et al. three commercial kits (Elecsys HBsAg II, Architect HBsAg and LiaisonXL Murex HBsAgQuant) were evaluated, and neither of these kits could detect two of the mutant strains. One of the strains was identified as genotype D and contained multiple substitutions including sG145R and the other was genotype E and contained two substitutions (sY100S / sL127P) (16).

Mutations on the second loop (aa 139-147) of the "a" determinant, including sG145R mutation in the literature, are often associated with low HBV DNA levels or HBV DNA levels that decrease over time (17, 18). In our case, HBV DNA load was found to be high levels as opposed to many other studies within the literature. rtV173L + rtA181G mutations may be responsible from this finding. These mutations have compensatory mutation (viral replication reparative, viral load enhancer) characteristics for nucleoside analogues and this might explain the high viral load measured in the patient, contrary to our expectations. However, more in-depth investigation and similar case series are required to fully express the clear relationship between them. On the other hand, the clinical effects of rtI169F, rtA181G and rtT184R mutations associated with nucleos(t)ide analogs detected in the *pol* gene are not yet known. Although rtV173L and rtA181G are known to be of compensatory nature for lamivudine, there are not sufficient studies that would explain that rtA181G has a similar effect on adefovir and telbivudine.

In the literature, some mutations in the preS/S region (sG145R, sM133T, etc.) are also associated with severe clinical forms such as fulminant hepatitis, cirrhosis and HCC (18, 19). In a study, 62 patients with HCC were examined and HBsAg mutation was detected in 20 patients, 17 of these were on different regions of "a" determinant. sM133T (transformation from methionine to threonine at 133th position) was determined in 9 cases, and sG145R mutation was detected in one case. Tumors in these patients were highly aggressive, usually diffuse and had multi-centric involvement (19).

In precore region mutations, while HBeAg is negative, anti-HBe and HBV DNA positivity can occur as an atypical serological profile (20). In our study, mutation analysis for this region could not be performed; therefore, we could not evaluate if precore region mutations accompanied the existing presentation.

In our case, histopathological verification and additional molecular investigations could not be performed because of the rapidly deteriorating general condition and ensuing loss of the patient in a short period of time, but history of HBV infection, AFP elevation and the imaging features of liver lesion were interpreted in favor of HCC. In the presence of sG145R mutation, HBsAg detection test used in our hospital (Elecsys HBsAg II) could not detect the virus and it escaped from the immune response despite anti-HBs positivity.

Conclusion

During the course of CHB infection, anti-HBs formation does not always imply development of a protective immune response, regardless of having HBsAg negativity or not. The presence of HBV DNA in the serum despite the formation of anti-HBs may be an indicator of S gene mutation. Mutant strains cause disease to progress without any notice, they can as well be transmitted by horizontal and vertical routes posing a threat to public health. Therefore, when atypical serological profiles are encountered, it is of utmost importance to remember the S gene mutations and perform necessary analyses. Meanwhile, we should pay attention to the fact that the capacity of the utilized kits should allow for detection of such HbsAg variants.

Abbreviations

HBV- hepatitis B virus
CHB- chronic hepatitis B
HBsAg- hepatitis B virus surface antigen
RT- reverse transcriptase
ORFs- open reading frames
USG- *ultrasonography*
ALT- alanine *aminotransferase*
NR- normal range
AST- aspartate aminotransferase
GGT- gamma-*glutamyltransferase*
ALP- alkaline phosphatase
PT- prothrombin time
INR- international normalized ratio
AFP- alpha-fetoprotein
HCV- hepatitis C virus
HIV- human immunodeficiency virus
HAI- *histological activity index*
MRI- magnetic resonance imaging
HCC- hepatocellular carcinoma
aa- aminoacid
HBIg- HBV immunoglobulin

Authors' contributions

SA; drafting the original manuscript, collecting data and literature searching. MTY; writing and critical revision of the manuscript. MS; performing molecular analysis, interpreting the results and editing the manuscript. SüA and MK; contributing to acquisition of data, editing the manuscript. All authors read and approved the final version of manuscript.

Conflict of interest

The authors declare no conflict of interests.

Ethics Committee Approval

This study was approved by the Local Ethics Committee (Okmeydani Training and Research Hospital (approval number:1281, 14.05.2019)

Informed Consent

Informed consent was obtained from patient and his guardian

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