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Original Article

Naturally Occurring Polymerase and Surface Gene Variants of Hepatitis B Virus in Turkish Hemodialysis Patients with Chronic Hepatitis B

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SUMMARY: The aim of this study was to assess the frequencies and patterns of naturally occurring genotypic resistance to nucleos(t)ide analogues (NUCs) and typical hepatitis B surface antigen (HBsAg) amino acid substitutions in naive hemodialysis (HD) patients with chronic hepatitis B. In order to achieve this, the genotypic resistance to NUCs and HBsAg amino acid substitutions were classified into primary/compensatory resistance mutation and antiviral drug-associated potential vaccine-escape mutation (ADAPVEM)/typical HBsAg amino acid substitution, respectively. Direct sequencing of polymerase (*pol*) gene of hepatitis B virus (HBV) was performed on DNA samples obtained from 248 HBsAg-positive Turkish patients. Overall, 38% (n = 94) of HBsAg-positive HD patients had detectable HBV DNA in their serum. Naturally occurring primary and compensatory resistance mutations to NUCs were detected in 30% (n = 28) and 52% (n = 49) of HD patients, respectively. However, 6 types of ADAPVEMs and 48 types of typical HBsAg amino acid substitutions were found in 10.6% (n = 10) and 46% (n = 43) of the HD patients, respectively. Our study suggests that every HD patient diagnosed with chronic hepatitis B, who is a potential candidate for NUCs treatment, should also be monitored for the baseline *pol* gene sequence changes before the initial treatment, for a more effective management of future treatment options. Further, a relatively higher frequency of ADAPVEMs variants needs to be addressed as a public health problem.

INTRODUCTION

Hepatitis B virus (HBV) infection in patients undergoing hemodialysis (HD) is a major concern as antibody response rates to HBV vaccination are low in these patients (1). However, owing to the lack of technical expertise, the currently available therapies—nucleos(t)ide analogues (NUCs) therapy and interferon-α—have not played a major role in the therapy of HBV infection in HD patients (1). As the HD process requires vascular access for prolonged periods, thus making them prone to intense immunosuppression compared to the general population, HD patients are at an increased risk of acquiring HBV. However, the prevalence of HBV infections in dialysis units of both developed as well as developing countries is low, with chronic hepatitis B surface antigen (HBsAg) seropositivity ranging from 0%–10% and 2%–22% in patients on long-term dialysis, respectively (2). The HD procedure presents an opportunity for the patients' blood to come in contact with contaminated equipment, injection syringes containing liquids harboring the virus, or via the exposure of broken skin or mucous membranes to infection; it also enables transmission of HBV among patients as well as between patients and staff members (3). Antiviral therapy of HBV necessitates dose adjustment in HD patients with chronic renal failure (4). Nonetheless, NUCs therapy for HBV-infected HD patients is complicated and has several side effects. Therefore, the management of treatment procedures for these patients should be based on the potential benefits and risks of therapy, prior to administration (5). NUCs therapy for HBV-infected HD patients remains unsatisfactory, although recent recommendations support the use of NUCs treatment in such patients (5,6).

In many parts of the world, five HBV-specific NUCs belonging to three subclasses, targeting the viral polymerase are approved for the treatment of chronic hepatitis B (CHB), namely, L-nucleosides (lamivudine [LAM] and telbivudine [LdT]), deoxyguanosine analogues (entecavir [ETV]), and acyclic nucleoside phosphonates (adefovir [ADV] and tenofovir [TDF]) (7). Emtricitabine (FTC) is available in Europe; however, in the United States, it is approved for human immunodeficiency virus (HIV) treatment only (4). Although HBV-specific NUCs decrease HBV DNA levels, they can also lead to resistance. A major concern involving NUCs treatment is the development of drug resistance in patients undergoing long-term therapy (8). Antiviral drug resistance is defined as the reduced susceptibility of a virus to the inhibitory effect of a specific drug. Two types of antiviral resistance mutations have been identified, namely, primary resistance mutations,

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which are directly responsible for the associated drug-resistance, and secondary or compensatory mutations, which increase the replicative capacity of the virus (9,10). In addition, it is important to keep in mind that the HBV polymerase (*pol*) gene completely overlaps with the envelope (S) gene (11). Mutations associated with NUCs treatment can cause changes to the surface antigen (HBsAg) protein of HBV, thus forming antiviral drug-associated potential vaccine-escape mutants (ADAPVEMs); therefore, antibodies directed against the HBsAg protein may not neutralize the virus (12). Consequently, these changes result in (i) the reactivation of HBV in previously anti-HBs immune persons, (ii) problems during diagnosis, and (iii) failure of infection prevention, either with vaccination or hepatitis B immunoglobulin (HBIg) (9).

Turkey is a country of intermediate/high endemicity for HBV infection, with the HBsAg prevalence in the general population ranging from 2.5%–9.1%, (especially high in the southeastern and eastern parts of Turkey) and depending on the region and the type of study (13). According to the general prevalence data at the end of 2009, the number of chronic HD patients, including pediatric patients, in Turkey was 48,433, and the HBsAg positivity rate was 4.4% (14). However, frequencies and patterns of HBsAg amino acid substitution related to the escape from anti-HBs neutralization and genotypic resistance to NUCs have not been reported in Turkish HD patients.

The objective of the present study was to determine the frequencies and patterns of naturally occurring genotypic resistance to NUCs and typical HBsAg amino acid substitutions in Turkish HD patients with CHB.

MATERIALS AND METHODS

Patient population: The present study was conducted between January 2010 and February 2011, and it included 248 HBsAg-positive patients receiving undergoing long-term HD in 41 HD centers from all the regions of Turkey. Out of the 248 serum samples examined, 18 were collected from Central Anatolia; 55, from Aegean; 88, from Marmara; 9, from the Black Sea; 16, from Eastern Anatolia; 25, from South-Eastern Anatolia; and 37, from the Mediterranean regions of Turkey. Clinic and laboratory characteristics of the HD patients are shown in Table 1. The patients had no history of intravenous drug use and renal transplantation. The demographic details and laboratory and clinical information status were provided by the clinical staff of the HD centers. The study was approved by the local ethics committee, and a written informed consent was obtained from each patient. All the patients who were undergoing HD were categorized as HBV chronic carriers

Table 1. Demographic and clinical features of the HBV DNA positive hemodialysis patients

Patients, no.	94
Gender, male/female	60/34
Age, median year (range)	60 (42–88)
Hemodialysis duration[1], median year (range)	5.7 (0.5–26)
HBeAg positive, no (%)	9 (9.5)
ALT, median U/L (range)	19 (5–77)
HBV DNA, median log IU/mL (range)	2.64 $\log_{10}$ (1.48–4.69)
HBV genotype[2], no. (%)	D: 93 (99) G[3] + A[4]: 1 (1)
Clinical status, no. (%)[5],[6]	
HBe antibody-negative	Patients in the ''immune tolerant'' phase: 9 (9.6)
HBe antibody-positive	Patients in the ''inactive HBV carrier'' phase: 68 (72.3) Patients in the ''HBeAg-negative CHB'' phase: 17 (18.1)
Co-infection status, no. (%)	
anti-HCV positive	12/94 (13)
anti-HDV IgG positive[7]	Not detected
anti-HIV positive	Not detected
Anti-HBV vaccine status, no. (%)	No vaccination

[1]: Hemodialysis duration was calculated from the date of the first hemodialysis procedure to the date of the collection of the sera.
[2]: HBV genotyping implemented as routinely in our clinical laboratory by phylogenetic analysis and based to reverse transcriptase (codon, 43–344) and S gene (codon, 34–277) regions of HBV sequencing (768 bp). In genotyping of HBV, phylogenetic comparison was performed by neighbor-joining analysis using the CLC Sequence Viewer 6.0.2 (CLC bio A/S, Aarhus, Denmark) software. However, HBV genotype was determined also by Genafor/Arevir–Geno2pheno Drug Resistance Tool (Center of Advanced European Studies and Research, Bonn-Germany, www.coreceptor.bioinf.mpi-inf.mpg.de).
[3]: GenBank accession no. is available, JN010438.
[4]: Genotype A of HBV has been detected at the time of confirmation of genotype G in Inno-Lipa HBV genotyping assay.
[5]: Clinical status categorized as per the European Association for the Study of the Liver clinical practice guidelines (7).
[6]: Three patients had interferon therapy experience in the previous year.
[7]: Anti-HDV IgG testing was available only for 36 hemodialysis patients.
HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; CHB, chronic hepatitis B; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus.

according to the European Association for the Study of the Liver (EASL) clinical practice guidelines, and they were treatment-naive (7). Blood samples were obtained prior to the HD session, centrifuged immediately, and the sera were separated, aliquoted, and then stored at −20°C until use. Serological markers of viral hepatitis B were measured by enzyme-linked immunosorbent assay (ELISA) (GBC kit; Gentaur BVBA and Genoprice, Brussels, Belgium) in all local HD units.

The study has been approved by the Clinical Research Ethics Committee of Kocaeli University (Project no., KKAEK 2009/24; Date, November 24, 2009; Approving no., 5/16).

HBV DNA detection: HBV DNA was isolated from all the serum samples on the Bio-Robot workstation using the magnetic-particle technology (QIAsymphony SP; Qiagen GmbH, Hilden, Germany). HBV DNA was detected and quantified by a commercial polymerase chain reaction (PCR) assay (artus HBV QS-RGQ test; Qiagen) on the real-time platform (Rotor-Gene Q; Qiagen).

HBV sequencing: Specific primer pairs were designed (forward: 5′-TCGTGGTGGACTTCTCTCAATT-3′ and reverse: 5′-CGTTGACAGACTTTCCAATCAAT-3′) for amplifying the HBV *pol* gene region (*pol* gene sequence was employed for HBV genotyping and genotypic resistance analysis to NUCs as a part of routine investigations in our clinical laboratory). The PCR conditions were applied as previously described (15).

Determination of HBV genotype and *pol*/surface gene mutation: The Genafor/Arevir-geno2pheno drug resistance tool (Center of Advanced European Studies and Research, Bonn, Germany [http://coreceptor.bioinf.mpi-inf.mpg.de/]) for HBV is a database that is specifically designed for rapid computer-assisted virtual phenotyping of HBV, and it accepts nucleic acid sequences as an input. Geno2pheno searches for homology between the entered sequences and those sequences that are stored in its database; it also stores relevant clinical data for HBV genotype, drug resistance, and S gene mutations. The Geno2pheno tool searches for HBV drug resistance mutations in the rt domain of the *pol* gene (8). Genotypic resistance mutations to the NUCs were classified as primary and compensatory categories (9). However, the overlapping S-gene segment was searched by the Geno2pheno tool (16). The

Table 2. Target region and amino acid position in the determination of hepatitis B virus polymerase and surface gene mutation

Target region	Amino acid position
Polymerase gene	
Reverse transcriptase gene segment	74, 80, 82, 84, 85, 139, 149, 156, 169, 173, 180, 181, 184, 194, 200, 202, 204, 214, 215, 233, 236–238, 250
Overlapping surface gene segment	100, 101, 105, 109–110, 114, 117–121, 123, 124, 126–135, 137, 139–142, 144–149, 151–153, 155–157, 161, 172, 173, 175, 176, 193–196
HBsAg protein	
ADAPVEM region	161, 164, 172, 173, 175, 176, 182, 193, 194, 195, 196
HBIg-selected escape mutation region	118, 120, 123, 124, 129, 133, 134, 144, 145
Vaccine escape mutation region	120, 126 133, 143–145, 193
Hepatitis B misdiagnosis mutation region	120, 131, 133, 143
Immune-selected mutation region	100, 101, 105, 109, 110, 114, 117, 119, 120, 123, 127, 128, 130–134, 140, 143–145

HBsAg, hepatitis B surface antigen; ADAPVEM, antiviral drug-associated potential vaccine-escape mutation; HBIg, hepatitis B immunoglobulin.

Table 3. Characteristics of genotypic resistance mutations to the nucleos(t)ide analogues in the treatment-naive hemodialysis patients

Mutation characteristic	Mutation pattern	Nucleos(t)ide analogue	Patient no. (%)
Primary resistance mutation	rtA181G/L/S/T	LAM, LdT, L-FMAU, ADV, TDF (PR)	5 (5.5)
	rtT184I/K/S	ETV (PR)	2 (2.2)
	rt194S/X/T	TDF (PR)	3 (3)
	rtS202N/R/T	ETV (PR)	3 (3)
	rtM204I/L/K/V ± rtV173L ± rtL180M	LAM, LdT, L-FMAU, FTC	10 (10.5)
	rtM204I/V + rtT184K/S	LAM, LdT, ETV	2 (2.2)
	rtI233V	ADV	1 (1)
	rtM250I/R	ETV (PR)	2 (2.2)
		Total	28 (30)
Compensatory mutation	rtQ149K	ADV	14 (15)
	rtL180V/R	LAM, L-FMAU, FTC, TDF	2 (2.2)
	rtV214A	LAM, L-FMAU, ADV, TDF	9 (10)
	rtQ215H/P/S	LAM, L-FMAU, ADV, TDF	19 (20)
	rtN238D/T	ADV	5 (5)
		Total	49 (52)

LAM, lamivudine; LdT, telbivudine; L-FMAU, clevudine; FTC, emtricitabine; ADV, adefovir; ETV, entecavir; TDF, tenofovir; PR, possible resistance.

HBsAg amino acid substitution were categorized into ADAPVEM and typical HBsAg amino acid substitutions, which included HBIg-selected escape, vaccine escape, hepatitis B misdiagnosis, and immune-selected amino acid substitutions (9,10,17–20). There were some mutations, especially ADAPVEMs, which were not located in the ''a'' determinant of the HBsAg protein. Further, we analyzed the important neutralizing domains of the HBsAg protein, which also included the region outside the ''a'' determinant of the HBsAg protein for ADAPVEMs. All the analyzed domains and their amino acid positions of HBV *pol* and surface genes (including typical HBsAg amino acid substitutions) are shown in Table 2.

RESULTS

In the present study, real-time PCR analysis confirmed that 94 of 248 (38%) HBsAg-positive HD patients had detectable HBV DNA in the serum (Table 1).

Naturally occurring primary resistance mutations to NUCs were detected 28 of 94 (30%) treatment-naive HD patients using the Geno2pheno tool database. In contrast, compensatory mutations were found in 49 of 94 (52%) patients. The frequencies and patterns of primary/compensatory resistance mutations in treatment-naive HD patients are shown in Table 3.

ADAPVEMs and typical HBsAg amino acid substitutions revealed by direct sequencing were detected in 10 (10.6%) and 43 (46%) of the 94 patients, respectively. Six types of ADAPVEMs and 48 types of typical HBsAg amino acid substitutions were detected at 26 amino acids positions corresponding to the overlapping S gene. ADAPVEM patterns and related NUCs, typical HBsAg amino acid substitution patterns, and related mutation types are shown in Tables 4 and 5.

A list of a few identified mutations—rtA181G/sW172C, rtA181S/sW172C + sL175S, rtM204K/sW196R, and rtM204L/sL176KQR—which were not previously recognized as ADAPVEMs but may be related to ADAPVEMs, are also shown in Table 4.

Table 4. Antiviral drug-associated potential vaccine-escape mutant (ADAPVEM) in the treatment naive hemodialysis patients

Mutation characteristic	Mutation pattern	Nucleos(t)ide analogue	Patient no. (%)
ADAPVEM	rtV173L/sE164D	LAM	1 (1)
	rtM204V/sI195M	LAM, LdT	4 (4.5)
	rtM204I/sW196L	LdT	1 (1)
	rtA181T/sW172L	ADV, TDF	2 (2)
	rtT184S/sL175F	ETV	1 (1)
	rtT184S/sL176V	ETV	1 (1)
		Total	10 (10.6)

Abbreviations are in Table 3.

DISCUSSION

Treatment of CHB patients undergoing HD with new potent antivirals as the first-line monotherapy (i.e., LdT and ETV oral solution available for individual dosage) of a high genetic barrier to HBV resistance, in doses modified according to their renal function, has been recently recommended (21). This necessitates antiviral treatment during the HD period for patients who are potential kidney transplant candidates, priot to kidney transplantation. However, patients with a history of HBV infection need to be closely monitored and be administered the treatment regimen accordingly, when

Table 5. Typical HBsAg amino acid substitution in the treatment-naive hemodialysis patients

HBsAg amino acid substitution category	Mutation pattern	Patient no. (%)	Combined pattern[1)]	Patient no. (%)
HBIg selected escape[2)]	sT118A/R, sP120K/Q/T, sT123A, sC124G, sQ129R, sM133L, sY134N, sD144E, sG145E/K/R	18 (19)	•sT118R + sG145E/K/R	1 (1)
Vaccine escape[3)]	sP120S, sT126I, sM133L, sS143L, sD144E, sG145R, sS193L	15 (16)	•sS143L + sG145R	1 (1)
Hepatitis B misdiagnosis[2)]	sP120S/T, sT131I, sM133T, sS143L	8 (8.5)	—	—
Immune-selected amino acid substitution[2)]	sY100C/S, sQ101H/R, sP105A/R, sL109R, sI110L, sS114A/T, sS117G/N, sG119I/R/V, sP120T, sT123A/D/N, sP127T, sA128V, sG130E/K/R, sT131N, sS132C/P, sY134F, sT140I, sS143T, sD144E, sG145R	31 (33)	•sY100C + sS117G + sG119I/R/V + sP120K/Q/T + sT123A/D/N	1 (1)
			•sP105A + sY134F	1 (1)
			•sP127T + sS143T	2 (2)
			•sY100S + sP105R + sA128V + sG130E/K/R	1 (1)
			•sY100C + sQ101H + sI110L	1 (1)
			•sT131N + sT140I	1 (1)
			•sS114A/T + sS132C	1 (1)
	Total[4)]	43 (46)	Total	10 (10)

[1)]: Combination pattern of HBs mutation in one genome. Each pattern was detected in a different patient.
[2)]: Reference no. 17 and no. 18.
[3)]: Reference no. 11.
[4)]: This value includes one typical HBsAg amino acid substitution for each patient.
HBIg, hepatitis B immunoglobulin.

viremia increases. Nontheless, in inactive HBV carriers, HBV recurrence and reactivation may be prevented by administering prophylactic antiviral therapy with LAM or other NUCs. But, for the efficacy of LdT, ETV, ADV, and TDF, extensive research is warranted (5). Although the treatment costs for renal impairment are low with drugs such as LAM, which are also available in the form of oral solutions for individual dosage, they cannot be relied upon owing to their susceptibility to resistance mutations. An additional factor when using LAM is the development of cross-resistance with other NUCs (i.e., LdT, ADV, and ETV) (4,21,22). The present study demonstrated that naturally occurring mutations in *pol* gene of HBV occur in clinical significance in treatment-naive HD patients infected with HBV. We detected naturally occurring primary and compensatory mutations to NUCs, in 30% and 52% of treatment-naive HD patients, respectively. Although most patients were identified to be hepatitis B e-antibody-positive asymptomatic carriers, they were found to be older in age with low hepatitis B e-antigen positivity, low alanine aminotransferase level, and low HBV DNA levels (Table 1). Our previous reports established that the frequencies of naturally occurring primary and compensatory mutations are very high in treatment-naive HD patients when compared to treatment-naive CHB patients with normal renal function (15,23). However, clinical manifestation and the number of viral genomic substitutions in HBV carriers may be influenced by aging under long-term pressure from the host immune system. Therefore, a comparison between treatment-naive HD and non-HD patients with regard to their age is necessary for discussing accurate frequencies and patterns of naturally occurring genotypic resistance to NUCs in treatment-naive HD patients. Emergence of natural mutations should be expected due to the HBV genome characteristics. The major causes of primary drug resistance and compensatory mutations include viral factors such as kinetics of viral production and clearance, and lack of proof-reading capacity during reverse transcription, which in turn creates a large HBV quasispecies pool and replication fitness of the latter (24). In a chronically infected individual, the extent of HBV replication is considerable, reaching $>10^{12}$ virions per day (25). Because *pol* is an rt that lacks the proof-reading capacity, HBV replication is thought to be associated with a high mutational rate of 10^{-5} substitutions/base/cycle (26), thus generating numerous single-base changes in the HBV genome daily; hence, these mutations are responsible for conferring resistance to NUCs in patients with existing HBsAg amino acid substitutions prior to therapy (27). In the present study, we determined naturally occurring mutations mostly related to L-nucleosides (which are currently approved NUCs employed for the treatment of CHB patients). Our previous study showed that naturally occurring mutations, which confer resistant to acyclic nucleoside phosphonates, were predominant in treatment-naive CHB patients with normal renal function (23).

Recently published studies have conferred a new acronym to these HBV *pol*/S gene overlap mutants: ADAPVEMs for antiviral drug-associated potential vaccine-escape mutants (12). The public health significance of ADAPVEMs was recently demonstrated in chimpanzees, when the theoretical concern of NUCs resistant-HBV behaving as a potential vaccine escape mutant (28). Our knowledge about clinical evidence for the spread or transmission of ADAPVEMs is limited (20). Infection by ADAPVEMs in human vaccines has not been reported yet; however, transmission of drug resistance-associated HBV mutants to non-vaccinated individuals has been reported, and the cases identified were primarily infected with a LAM-resistance associated rtM204V/sI195M mutant. Nonetheless, it is unknown if the infecting HBV variants was naturally occuring (29). The present study is the first of its kind to report a high incidence (10.6%) of ADAPVEMs in HD patients, where 0.7% of ADAPVEMs occurred in CHB patients with normal renal function (30). All the patients harboring ADAPVEMs were treatment-naive (Table 4), indicating that ADAPVEMs in these patients occurred either naturally or were transmitted from another patient infected with HBV. To gain further insights into this mechanism, a clear understanding about ADAPVEMs, their transmissibility, and pathogenicity is necessary, which could be possibly achieved by virological surveillance, clinical follow-up of infected individuals and those undergoing treatment, and surveillance of their close contacts (12). The detected ADAPVEMs in HD patients were related to all approved NUCs in this study. However, the selection of NUCs was limited to L-nucleosides (LAM and LdT) and acyclic phosphonates (ADV) in CHB patients with normal renal function. Some HD patients had atypical substitutions such as rtA181G/sW172C (related to L-nucleosides and acyclic phosphonates), rtA181S/sW172C + sL175S (related to L-nucleosides and acyclic phosphonates), rtM204K/sW196R (related to L-nucleosides), and rtM204L/sL176KQR (related to L-nucleosides). The occurrence of these atypical substitutions may be related to new mutations for ADAPVEMs. However, clear interpretation of the results is limited by the presence of insufficient data. It is well known that LAM is a relatively inexpensive and obligatory drug that is used during the initiation of HBV treatment in Turkey. In populations where LAM has been widely used to treat patients over several years, viruses with alterations in the S gene are likely to occur relatively frequently, and some would possibly be ADAPVEMs. Therefore, idenfying the pre-existing ADAPVEMs may be necessary to select an appropriate drug regimen at the beginning of CHB treatment in HD patients. Further, vaccination against HBV is recommended for all HD patients and staff members of all dialysis units. As renal failure is associated with a poor response to HBV vaccination, double-dose vaccines have been approved for use in this patient group (1). Nonetheless, the emergence of ADAPVEMs may present a risk to the local and/or global hepatitis B immunization program. Recently published reviews reported that ADAPVEMs have opportunities to spread to immunized individuals through hepatitis B vaccination (12,19).

Several previous studies identified typical HBsAg amino acid substitutions are sP120T, sM133I, sS143L, sD144A/E, sG145R, sE164D, sW172*, and sW182* which is the most commonly occurring pattern (9,15,16). Vaccine escape/HBIg-selected escape mutants, sG145R and sP120T, in combination with LAM-

associated resistance mutations, are often seen in HBV mono-infected patients after LAM or HBIg treatment (31). The mutation selected during NUCs resistance can cause concomitant changes to the overlapping reading frame, resulting in major resistance mutations for LAM, ADV, and ETV—in particular, altering the C-terminal region of HBsAg (32). The typical primary mutation associated with LAM-resistant HBV (rtM204V/sI195M) and compensatory mutation (rtV173L/sE164D) have significantly reduced anti-HBs antibody binding (vaccine-associated) because of changes in the HBsAg (11). The present study is also the first of its kind to report typical HBsAg amino acid substitutions in Turkish HD patients infected with CHB. Some of the detected mutations (sP120T, sG130R, sM133L/T, sY134N, sD144E, sS143L, and sG145R) have been shown to cause diagnostic problems in HBsAg assays and vaccine or HBIg therapy escape (16). However, insufficient data is available for interpretation, and the clinical effects of some of the detected typical HBsAg amino acid substitutions found in this study (i.e., sY100C/S, sL109R, sI110L, sS117G/N, sP127T, and sS132C/P) are not clear in the background literature (16). Published reports related to naturally occurring mutations in the HBV *pol* and/or S genes in the renal failure patients are limited. A recent report demonstrated that multiple amino acids substitutions (at positions; 118, 120, 126, 127, 130, 134, 144, and 160), around and within the HBsAg ''a'' determinant in the isolates closely related to the HBV genotype D, can lead to escape from immune response to HBV vaccination (33).

Taken together, our results showed various naturally occurring rt sequence changes related to NUCs and typical HBsAg amino acid substitutions in overlapping S gene in treatment-naive Turkish HD patients with CHB. Further, our study suggests that every HD patient diagnosed with CHB, who is a potential candidate for NUCs treatment, should also be monitored for baseline rt sequence changes before initial treatment, for a more effective management of future treatment options. Our study detected the ADAPVEMs for the first time in Turkish HD patients and confirmed that treatment with NUCs in CHB may have a potential risk for the emergence of the ADAPVEMs. In addition to its transmission through hepatitis B vaccination, ADAPVEMs may spread within the immunized or non-immunized HD patients. Furthermore, a higher frequency of ADAPVEMs variants needs to be addressed as a serious public health problem. Understanding the public health risk of ADAPVEMs is an essential requisite and should be carefully monitored in infected HD patients, as these findings may have potential implications for the management of HD patients.

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Conflict of interest None to declare.

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