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Association of Hepatitis B Virus Polymerase/Surface Gene Overlap Mutations Developed in Nucleos(t)id Analogue Treatments of Chronic Hepatitis B with Hepatocellular Carcinoma

Kronik Hepatit B'nin Nükleoz(t)id Analoğu Tedavilerinde Gelişen Hepatit B Virüsü Polimeraz/Yüzey Geni Çakışma Mutasyonlarının Hepatosellüler Karsinom ile İlişkisi

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ABSTRACT

Aim: Nucleos(t)id analogs (NA) used in hepatitis B virus (HBV) treatment may cause rtA181T and sW172* mutations. sW172* increases progression to hepatocellular carcinoma (HCC). This study aimed to reveal the rtA181T/sW172* mutation in HCC patients on the background of chronic hepatitis B (CHB) to determine its association with NA.
Material and Methods: A total of 90 CHB patients, 42 patients with HCC (DNA of 4 patients could not be analyzed), and 48 patients without HCC as the control group, were included in this study. Patients in the control group were divided into two groups, those who received NA treatment (n=21) and those who did not (n=21). Drug resistance analysis was performed by DNA sequencing.
Results: Among the 42 patients with HCC, the median age was 63 (range, 37-81) years, and the median HBV DNA level was $6.0x10^6$ (range, 30-$1.14x10^8$) IU/ml. In the 48 patients included as controls, the median age and HBV DNA level were 46 (range, 20-75) years and $1.41x10^7$ (range, 80-$1.70x10^8$) IU/ml in the treatment-naive patients, and 36 (range, 21-50) years and $7.6x10^6$ (range, $15.9x10^2$ - $5.1x10^7$) IU/ml in the NA-treated patients, respectively. sW172* mutation, which causes rtA181T mutation to occur, was identified in only 2 (5.3%) patients in the study group with HCC.
Conclusion: Association between HCC and rtA181T/sW172* mutation suggests that HCC may develop in patients under NA treatment. The rtA181T/sW172* mutation should be screened in patients receiving treatment, and when detected, they should be closely monitored for HCC development.
Keywords: Hepatocellular carcinoma; hepatitis B virus; drug resistance; nucleos(t)id analog.

ÖZ

Amaç: Hepatit B virüsü (HBV) tedavisinde kullanılan nükleoz(t)id analogları (NA), rtA181T ve sW172* mutasyonlarına neden olabilir. sW172* hepatosellüler karsinoma (hepatocellular carcinoma, HCC) ilerlemesini artırır. Bu çalışmanın amacı, kronik hepatit B (chronic hepatitis B, CHB) zemininde HCC hastalarında rtA181T/sW172* mutasyonunu ortaya koyarak NA kullanımı ile ilişkisini belirlemektir.
Gereç ve Yöntemler: Bu çalışmaya, 42 HCC'li hasta (4 hastanın DNA'sı analiz edilemedi) ve kontrol grubu olarak 48 HCC'siz hasta olmak üzere toplam 90 CHB hastası dahil edildi. Kontrol grubundaki hastalar, NA tedavisi alanlar (n=21) ve almayanlar (n=27) olmak üzere iki gruba ayrıldı. İlaç direnci analizi DNA sekanslama yöntemi ile gerçekleştirildi.
Bulgular: HCC'li 42 hastada, ortanca yaş 63 (aralık, 37-81) yıl ve ortanca HBV DNA düzeyi $6,0x10^6$ (aralık, 30-$1,14x10^8$) IU/ml idi. Çalışmaya kontrol olarak dahil edilen 48 hastada, ortanca yaş ve HBV DNA düzeyi, sırasıyla, tedavi naif hastalarda 46 (aralık, 20-75) yıl ve $1,41x10^7$ (aralık, 80-$1,70x10^8$) IU/ml ve NA tedavisi alan hastalarda 36 (aralık, 21-50) yıl ve $7,6x10^6$ (aralık, $15,9x10^2$ - $5,1x10^7$) IU/ml idi. Çalışma grubundaki HCC'li hastaların sadece 2'sinde (%5,3), rtA181T mutasyonunun ortaya çıkmasına neden olan sW172* mutasyonu tespit edildi.
Sonuç: HCC ile rtA181T/sW172* arasındaki ilişki, NA tedavisi alan hastalarda HCC gelişebileceğini düşündürmektedir. Tedavi alan hastalarda rtA181T/sW172* mutasyonu taranmalı ve tespit edildiğinde hastalar HCC gelişimi açısından yakın takip edilmelidir.
Anahtar kelimeler: Hepatosellüler karsinom; hepatit B virüsü; ilaç direnci; nükleoz(t)id analoğu.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common hepatic malignancies and the sixth most common cancer worldwide (1). Hepatitis B virus (HBV) is a risk factor for HCC. 20-30% of adults with chronic hepatitis B (CHB) will develop cirrhosis and/or HCC (2). The molecular processes underlying HCC development are complex and various, either independent of liver disease etiology or etiology-related. The reciprocal interlinkage among non-viral and viral risk factors, the damaged cellular microenvironment, the dysregulation of the immune system, and the alteration of gut-liver-axis are known to participate in liver cancer development and progression (3). HBV genome, due to its ability to integrate into the host, can cause genomic instability, disrupting normal regulatory mechanisms (4).

HBV genome is a circular, double-stranded DNA virus that contains four readable gene regions (ORFs). Gene regions encode envelope (pre-S/S/S), core (pre-core/core), pol, and X protein. Pre-S/S/S region synthesizes L, M, and S surface glycoproteins. The pre-core/core region encodes nucleocapsid core protein and synthesizes HBeAg. The pol gene encodes a DNA polymerase, which is reverse transcriptase (RT) (5). HBx protein is a multifunctional regulator, and X protein plays an essential role in the progression of HCC (6).

X protein modulates cellular transcription, protein degradation, proliferation, and apoptotic signaling pathways; it does not directly bind to DNA but affects cells by altering protein-protein interactions and cytoplasmic signaling pathways (7).

Nucleos(t)id analog (NA) used in CHB treatment causes point mutations. These mutations may cause drug resistance (8). Drug resistance is classified into two groups as primary resistance and compensatory mutations. Primary drug resistance mutations mainly lead to non-responsiveness, and compensatory mutations repair replication ability due to the circular structure of HBV pol and S gene overlap (9). This overlapping (pol/S) leads to changes in the region encoding HBsAg protein in primary/compensatory drug resistance mutations. According to findings obtained in recent years, it leads to the formation of potential vaccine escape mutations, namely antiviral drug-associated potential vaccine escape mutation (ADAPVEM) (10).

Lamivudine (LMV) and adefovir (ADV) are safe and inexpensive for long-term use in the treatment of CHB. For this reason, it is used widely and for a long time. Resistance to these drugs develops as a result of long-term use (11). Due to the circular structure of HBV, gene loci overlap each other. As a result of this overlap, point mutations occur. The main one is rtA181T (major drug resistance mutation) on the pol gene, causing sW172* mutation on the S gene (9). The stop codon (sW172*) mutation causes large pieces from the C-terminal hydrophobic area to be cut off and these pieces to accumulate in the cell. L, M surface proteins accumulating within the cell cause stress in the endoplasmic reticulum, leading to progression to HCC (12).

The goal of this study was to indicate the presence of the rtA181T/sW172* mutation in patients who developed HCC at the CHB level and reveal the relationship of these mutations with NA use.

MATERIAL AND METHODS

Forty-two patients over 18 years with HCC and who were admitted to the Gastroenterology Department of the Kocaeli University Faculty of Medicine between 2012 and 2014 were included in the study. Patients were diagnosed by the combination of serologic markers, imaging techniques, and/or biopsy. Serum from 19 patients and biopsy material from 23 patients were used. HBV, hepatitis C virus (HCV), human immunodeficiency virus (HIV), hepatitis D virus (HDV) markers, and HBV DNA were analyzed. Patients with positive HBV DNA levels were included (n=38). Biopsy materials constituting the other part of the study group were obtained from the Pathology Department of the Kocaeli University Faculty of Medicine. As the control group, 48 patients with CHB were divided into two groups as treatment naive (n=27) and patients receiving NA treatment (n=21). The study was conducted with the decision of the Kocaeli University Clinical Research Ethics Committee (2012/67).

Patient history was obtained, including their age, gender, smoking and alcohol use, as well as whether they were receiving antiviral treatment and the duration of the treatment. The presence and staging of cirrhosis were assessed, and the classification of HCC was determined based on clinical conditions and imaging methods. A 5 cc blood sample was collected and serum was separated by centrifugation and stored at -20 ºC. Patient files with pathologic materials were scanned, and information regarding age, gender, alcohol use, antiviral treatment, cirrhosis status, HCC staging, and clinical status was examined. Serum and pathology materials were analyzed using the HBV DNA sequencing method.

Hepatitis B Virus DNA Isolation

HBV DNA was isolated on the QIAsymphony SP magnetic particle isolation platform (QIAGEN GmbH, Hilden, Germany). Paraffin block sections were analyzed using the DNA spin column isolation technique (DNA MiniElute, QIAGEN GmbH, Hilden, Germany).

Real-Time Polymerase Chain Reaction

HBV DNA was analyzed by real-time polymerase chain reaction (PCR) using an RGQ kit on the Rotor-GENE platform (QIAGEN GmbH, Hilden, Germany) (13). HBV genotype detection, all known primary/compensatory NA resistance mutations, S gene (HBsAg protein; amino acids 111 to 227) mutations overlapping with pol gene were analyzed by sequencing HBV pol gene (RT region, amino acids 80 to 250), removal of paraffin with xylol (14).

HBV DNA was isolated from the serum sample (Anatolia Geneworks, Bosphore® Viral DNA Extraction Spin Kit, and Magnesia® 16 Magnetic Bead Extraction System, İstanbul, Türkiye). For HBV pol gene (742 bp) amplification, forward (F:5'-tcgtggtggacttctctctcaatt-3'), reverse (R:5'-cgttgacagactttccaatcaat-3') primers were used. PCR conditions were pre-denaturation at 95 °C for 10 minutes, followed by 35 cycles of 95 °C for 45 seconds, 60 °C for 45 seconds, and 72 °C for 45 seconds. PCR products were purified with a High Pure PCR Product Purification Kit (Roche Diagnostics, Germany). Phire Hot Start DNA polymerase (Finnzymes Oy, Finland) was used in the sequencing protocol. Sequencing was performed on the ABI PRISM 3130 (Applied Biosystems Inc, USA) platform using BigDye Terminator v3.1 Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc, USA), 36 cm

capillary and POP-7 TM polymer (Applied Biosystems Inc, USA) according to manufacturer's recommendations. PCR protocol used for direct sequencing was 35 cycles of 95 °C for 20 seconds, 50 °C for 25 seconds, and 60 °C for 2 minutes. Electropherograms were obtained using Vector NTI v5.1 (InforMax, Invitrogen, Life Science Software, USA). Sequences were analyzed in the Geno2pheno Drug Resistance program (Center of Advanced European Studies and Research, Germany).

The Geno2pheno program compares unknown nucleic acid sequences in FASTA format with reference sequences found in the database. After comparison, 80th, 84th, 85th, 91st, 169th, 173rd, 180th, 181st, 184th, 191st, 194th, 202nd, 204th, 214th, 215th, 233rd, 236th-238th, and 250th amino acid positions in HBV pol gene RT coil were analyzed for primary drug resistance and compensatory mutations. In addition, 121st, 135th, 137th, 139th-149th, 151st-153rd, 155th-157th, 161st, 164th, 172nd, 173rd, 175th, 176th, 182nd, and 193rd-196th amino acid positions in the S gene region overlapping with the HBV RT strand were analyzed for mutations (15).

Statistical Analysis

For statistical analysis, IBM SPSS version 20.0 was used. Following the normal distribution assumption, the student t test was used to compare groups as a parametric test, and the Mann-Whitney U test was used as a non-parametric one. Chi-square test was used to analyze categorical data. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 90 patients were divided into three groups. Patients with HCC (n=42) were included in the study group, and patients with CHB without HCC (n=48) were included in the control group. The control group was further divided into two groups as those receiving NA treatment (n=21) and those not receiving (n=27). Diagnosis of HCC was made by biopsy in 23 patients by combining alpha fetoprotein (AFP) value and HCC typical appearance in 2 imaging modalities in 19 patients. HBV DNA sequencing analysis could be performed in 38 (90.5%) patients in the study group. The majority of patients in the study groups were male, the mean age was higher in the HCC group compared to other groups (Table 1). When the HbeAg status of these patients was compared, no significant difference was found (p=0.478). Patients did not have superinfection/co-infection.

Information on AFP values, the presence of cirrhosis, and the alcohol intake status of patients with HCC was shown in Table 1. The majority of patients with cirrhosis and HCC were found to be C in Child-Pugh staging.

Table 1. Demographic information of the patients included in the study

	Study Group (n=42)	**No Treatment (n=27)**	**NA Treatment (n=21)**
Gender, n (%)			
Male	38 (90.5)	17 (63.0)	14 (66.7)
Female	4 (9.5)	10 (37.0)	7 (33.3)
Age (years), median (range)	63 (37 - 81)	46 (20 - 75)	36 (21 - 50)
AST (U/L), median (range)	142 (20 - 968)	41 (18 - 41)	135 (11 - 1042)
ALT (U/L), median (range)	179 (20 - 1021)	48 (18 - 48)	182 (17 - 1315)
HBeAg[*1] (+), n (%)	10 (25.0)	3 (11.1)	6 (28.6)
HBV DNA (IU/ml), median (range)	$6.0x10^6$ ($30 - 1.14x10^8$)	$1.41x10^7$ ($80 - 1.70x10^8$)	$7.6x10^6$ ($15.9x10^2 - 5.1x10^7$)
HBV genotype[*2] (D), n (%)	38 (100)	27 (100)	21 (100)
HBV sub genotype[*2], n (%)			
D1	35 (92.1)	26 (96.3)	17 (81.0)
D2	-	1 (3.7)	2 (9.5)
D3	3 (7.9)	-	2 (9.5)
AFP (ng/ml), median range	20025 (1-521300)	-	-
Cirrhosis[*3] (+), n (%)	32 (82.1)	-	-
Child-Pugh Staging, n (%)			
A	3 (9.4)	-	-
B	9 (28.1)		
C	20 (62.5)		
Treatment[*4], n (%)	18 (42.9)	-	21 (100)
Treatment[*4], n (%)			
LAM	11 (61.0)	-	12 (57.1)
ADV	1 (5.6)	-	5 (23.8)
LdT	1 (5.6)	-	1 (4.8)
ETV	1 (5.6)	-	3 (14.3)
LAM+ADV	2 (11.1)	-	-
LAM+TDF	2 (11.1)		-
Duration (month), median (range)			
LAM	43 (1 - 96)	-	26 (12 - 48)
ADV	32 (12 - 60)	-	20 (8 - 36)
LdT	1	-	9
ETV	12	-	18 (12 - 24)
TDF	90 (84 - 96)	-	-

NA: nucleos(t)id analog, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HBeAg: hepatitis B e antigen, HBV: hepatitis B virus, DNA: deoxyribonucleic acid, AFP: alpha fetoprotein, LAM: lamivudine, ADV: adefovir; LdT: telbivudine; ETV: entecavir; TDF: tenofovir, [*1]: no information on HBeAg status in two patients, [*2]: HBV DNA sequencing analysis could be performed in 38 patients, [*3]: no information on cirrhosis status in three patients, [*4]: no information on medication use in three patients

Patients were classified according to drug use. The HBV pol gene region was analyzed for drug resistance mutations in the study and the control group. Primary drug resistance, partial drug resistance, and compensatory mutations were analyzed. Primary drug resistance mutations (rtM204I/V, rtA181T, rtA184V, rtT184A/S) were detected in the study group and the NA control group, and were frequently associated with NA. No primary drug resistance mutations were detected in the treatment-naive control group.

Mutations causing primary drug resistance (rtM204I, rtA181T, rtA184V, rtT184S) were detected in 8/38 (21.1%) of the HCC group. In the control group, drug resistance mutations (rtM204I, rtM204V, rtT184A) were detected in 6/21 (28.6%) of the NA-treated group. No primary drug resistance was found in the treatment-naive group. When the groups were compared in terms of the primary drug resistance, no significant difference was found (p=0.833).

In the control group, partial drug resistance mutations were associated with NA. In contrast to primary drug resistance mutations, partial resistance mutations were found in the treatment-naive control group. Replication capacity restoring, viral load increasing mutations, and compensatory mutations (L91I, Q215H/S, Q149K) were detected in the study and control groups (Table 2).

The potential of oral antivirals used in the treatment of CHB to create vaccine escape mutations (ADAPVEM) was analyzed (Table 3). While no ADAPVEM patterns were detected in the treatment-naive control group, ADAPVEM patterns were detected in the study group and the control group under NA treatment. While sS143L, sW196L, and sW172* were detected in the study group, sI195M and W196L patterns were detected in the control group under treatment. The sW172* ADAPVEM detected in the study group is also the mutation that causes HBsAg to accumulate in the cell after truncation (Table 3). The W172* development rate between patients in the study group and the control group was not statistically significant (p=0.192). When the duration of drug use in both groups (HCC and NA treatment groups) was compared, no statistically significant difference was found between them (p=128 for LAM, p=0.252 for ADV).

Table 2. HBV polymerase gene mutations and drug resistance status in study and control groups

Mutation Pattern	Study Group* (n=42)	No Treatment (n=27)	NA Treatment (n=21)
Primary Drug Resistance, n (%)			
M204I	4 (10.5)	-	3 (14.3)
M204V	-	-	2 (9.5)
A181T	2 (5.3)	-	-
A184V	1 (2.6)	-	-
T184A	-	-	1 (4.8)
T184S	1 (2.6)	-	-
Partial Drug Resistance, n (%)			
A194S	1 (2.6)	-	-
A194X	-	1 (3.7)	-
C233F	1 (2.6)	-	-
P236T	1 (2.6)	-	-
L180M	1 (2.6)	-	5 (23.8)
Compensatory Mutation, n (%)			
L91I	2 (5.3)	-	4 (19.1)
Q215H/S	9 (23.7)	-	5 (23.8)
Q149K	1 (2.6)	1 (3.7)	3 (14.3)

HBV: hepatitis B virus, NA: nucleos(t)id analog, *: HBV DNA sequencing analysis could be performed in 38 patients

Table 3. Potential vaccine escape mutations associated with oral antivirals in the study and control groups

Mutation Pattern	Study Group* (n=42)	No Treatment (n=27)	NA Treatment (n=21)
ADAPVEM, n (%)			
I195M	-	-	2 (9.5)
S143L	3 (7.9)	-	-
W196L	4 (10.5)	-	3 (14.3)
W172*	2 (5.3)	-	-

NA: nucleos(t)id analog, ADAPVEM: antiviral drug-associated potential vaccine escape mutation, *: HBV DNA sequencing analysis could be performed in 38 patients

DISCUSSION

Global distribution of HCC depends on regions, incidence rate, gender, age, and etiology (16). The prevalence of HBV RT mutations prior to treatment varies. The ratio of HCC incidence in the non-cirrhotic and cirrhotic stages in HBV-related liver disease is 0.3-3.23% (17). These controversial mutations depend on geographic factors, HBV genotypes, HBeAg serostatus, HBV viral loads, and disease progression (18).

The median age of 42 HCC patients included in the present study was found to be higher compared to the control group. Studies have reported that 80-90% of patients with HCC have cirrhosis. A cohort study conducted in Italy showed that patients with stages B and C, according to Child-Pugh staging, had a higher risk of developing HCC compared to stage A (19). In the present study, the rate of cirrhosis in patients with HCC was similarly high (82.1%). When HCC patients with cirrhosis, a high proportion of patients were found to be in the Child C stage (62.5%).

Studies have shown that basal loads of HBV DNA are important in progression to HCC (20). In the present study, the median HBV DNA load was found to be higher in the treatment-naive group. This finding may be due to the suppression of HBV DNA loads after antiviral drug use in the study group and the group receiving NA.

The long-term use of NA therapy can result in drug resistance, which can lead to treatment failure (21). It has been shown that primary drug resistance developed in CHB treatment increases the risk of HCC (8). One study reported that HCC developed in 8 of 141 HCC patients with sL21*, sW156*, and sW172* mutations. This study may be important in showing that HCC develops after the use of LAM (8,20).

In another study conducted in South Korea between January 2004 and December 2012, it was reported that the risk of HCC development increased in patients who developed drug resistance. Cumulative risk of HCC development in cirrhotic patients who developed drug resistance in years 1, 2, 3, and 5 was found to be 3.93%, 5.55%, 6.71%, and 9.02% (22).

In a study involving 1948 CHB patients, 917 patients were found to have a drug-resistance gene mutation in the RT region (47.07%). HBV-related cirrhosis rate and HBV DNA load were higher in the group with drug resistance

than in the group without mutation. In patients with drug resistance, 53 gene mutation patterns were found. Among these patterns, rtL180M+rtM204V+rtS202G (9.70%) was the most dominant pattern. Five types of drug resistance were detected, the highest being LAM+Ldt (21.25%). Among 18 regions associated with LAM, ADV, ETV, and Ldt resistance in HBV RT regions, 14 regions were found to be mutated, and the most common mutation regions were rtL180M, rtM204V, rtM204, and rtS202G (23).

In the present study, mutations causing primary drug resistance (rtM204I, rtA181T, rtA184V, rtT184S) were identified in 8/38 (21.1%) of HCC patients. Mutations causing drug resistance (rtM204I, rtM204V, rtT184A) were identified in 6/21 (28.6%) of the group receiving NA. When groups using NA were compared, no statistically significant difference was found in terms of prevalence of primary resistance. However, the A181T mutation was identified in the HCC patient group (2/38, 5.3%), which was not found in the NA-treated control group. In HCC patients with the A181T mutation, the W172* mutation in the S gene. The median duration of LAM and ADV treatments, which are known to be responsible for the development of the rtA181T mutation, was 43 and 32 months in the HCC patient group and 26 and 20 months in the NA-treated group. The rate of W172* development between the study and control group was not statistically significant. When the duration of drug use was compared in HCC and NA groups, the difference was not statistically significant. Development of the A181T mutation may be independent of the duration of LAM and ADV use. This data shows that HBsAg escape mutations with epidemiologic and clinical significance can develop due to NA.

In another study, 100 patients who had been undergoing NA therapy for one year or more were included. 84 patients that could be sequenced with HBV pol gene HBV, 53 (63.09%) were males and 31 (36.91%) were females, and the mean age was 47±14.99 (range, 20-67) years. Primary/secondary drug mutations (rtM204I/V, rtI169S, rtL180M, rtT184L, rtA194V, rtM204I/rtL91I, rtQ149K, rtQ215H/S, rtN238D) were detected in 38 (45.2%) of the patients. Because of the HBV pol/S gene overlapping, in 27 patients immune-selected amino acid substitutions (sI110L, sT127P, sS114A, sT123A), in nine patients HBIg selected escape mutants (sP120R, sT123N, sE164D, sY134F, sQ129H, sT118A, sP127K), in seven patients vaccine escape mutants (sT126I, sP120S, sG145A, sS193L), and in one patient misdiagnosis of HBsAg (sT131I) were detected. In addition, ADAPVEM in 13 (15.4%) patients (20).

In a study, 6 types of ADAPVEM (sE164D, sI195M, sW196L, sW172L, sL175F, s176V) were identified in 10/94 (10.6%) patients (24). In another study, seven different ADAPVEM (rtM204V/sI195M, rtM204I/sW196S, rtM204I/sW196L, rtV173L/sE164D, rtA181T/sW172*, rtA181T/sW172L, and rtA181V/sL173F) mutations were found in 46/442 (10.4%). In this study, the rate of ADAPVEM in NA-treated and treatment-naive patients was found to be 44/186 (23.7%) and 2/256 (0.78%), respectively, and was reported statistically significant (24). The present study identified four different ADAPVEM. While 7/38 (18.4%) ADAPVEM were detected in the study group (sS143L, sW196L, sW172*), 5/21 (23.8%) were detected in the NA-treated control group (sW196L, sI195M). No ADAPVEM was found in the treatment naive control group. As in similar studies, ADAPVEM was observed only in NA-treated patients in this study. These findings suggest that NA treatment plays a role in the occurrence of ADAPVEM. sW172* causes accumulation of HBsAg in hepatocytes was detected only in 2/38 (5.3%) patients in the HCC group. In these patients, HBsAg accumulation in hepatocytes may be effective in HCC progression.

In another study, it was reported that drug resistance was detected in 36/198 (18.2%) of patients in whom NA was initiated in 320 decompensated cirrhotic patients. Among these patients, HCC was detected in 5 patients who did not respond to salvage therapy, and it was reported that the rtA181T mutation was associated with salvage therapy failure. However, since S gene analysis was not performed in this study, it was not stated whether the rtA181T leads to sW172*. However, according to the study, NA drug resistance plays a role in the cumulative incidence of HCC (24). Results of both studies indicate that patients should be followed up in the long term.

Major limitations of this study include a relatively small sample size, lack of follow-up from the initiation of the treatment, and lack of timing of HCC diagnosis.

CONCLUSION

The rtM204I, rtA181T, rtA184V, and rtT184S mutations causing primary drug resistance were found in 21.1% of HCC patients. In the control group, rtM204I, rtM204V, and rtT184A mutations were found in 28.6% of patients receiving NA. The relationship between HCC development and the rtA181T/sW172* mutation duo in CHB patients under NA treatment may be assessed in a larger study group with longer patient follow-up for more certain findings.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Kocaeli University (20.03.2012, 67).

Conflict of Interest: This manuscript was derived from the specialty thesis of the first author, Seda Erem Basmaz, and all authors declare that there is no conflict of interest.

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All authors were working at Kocaeli University during the study period, and were appointed to a different city, which reflects their current place of employment.

REFERENCES

1. Fidan E, Fidan S, Merev E, Kazaz N. The relationship between albumin-bilirubin grade and survival in hepatocelluler carcinoma patients treated with sorefanib. Nijer J Clin Pract. 2022;25(2):173-7.
2. Kumar R. Review on hepatitis B virus precore/core promoter mutations and their correlation with genotypes and liver disease severity. World J Hepatol. 2022;27(4):708-18.
3. Stella L, Santopaolo F, Gasbarrini A, Pompili M, Ponziani FR. Viral hepatitis and hepatocellular carcinoma: From molecular pathways to the role of clinical surveillance and antiviral treatment. World J Gastroenterol. 2022;28(21):2251-81.
4. Perisetti A, Goyal H, Yendala R, Thandassery RB, Giorgakis E. Non-cirrhotic hepatocellular carcinoma in chronic viral hepatitis: Current insights and advancements. World J Gastroenterol. 2021;27(24):3466-82.
5. Fang ZL, Sabin CA, Dong BQ, Ge LY, Wei SC, Chen QY, et al. HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. Am J Gastroenterol. 2008;103(9):2254-62.
6. Motavaf M, Safari S, Saffari Jourshari M, Alavian SM. Hepatitis B virus-induced hepatocellular carcinoma: the role of the virus X protein. Acta Virol. 2013;57(4):389-96.
7. Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. J Virol. 2004;78(23):12725-34.
8. Warner N, Locarnini S. Can antiviral therapy for chronic hepatitis B enhance the progression to hepatocellular carcinoma? Antivir Ther. 2009(14):139-42.
9. Locarnini S. Primary resistance, multidrug resistance, and cross-resistance pathways in HBV as a consequence of treatment failure. Hepatol Int. 2008;2(2):147-51.
10. Sheldon J, Rodès B, Zoulim F, Bartholomeusz A, Soriano V. Mutations affecting the replication capacity of the hepatitis B virus. J Viral Hepat. 2006;13(7):427-34.
11. Lai MW, Yeh CT. The oncogenic potential of hepatitis B virus rtA181T/surface truncation mutant. Antivir Ther. 2008;13(7):875-9.
12. Qi X, Xiong S, Yang H, Miller M, Delaney WE 4th. In vitro susceptibility of adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. Antivir Ther. 2007;12(3):355-62.
13. Kim JH, Jung YK, Joo MK, Kim JH, Yim HJ, Park JJ, et al. Hepatitis B viral surface mutations in patients with adefovir resistant chronic hepatitis B with A181T/V polymerase mutations. J Korean Med Sci. 2010;25(2):257-64.
14. Bock CT, Tillmann HL, Torresi J, Klempnauer J, Locarnini S, Manns MP, et al. Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. Gastroenterology 2002;122(2):264-73.
15. Avellón A, Echevarria JM. Frequency of hepatitis B virus 'a' determinant variants in unselected Spanish chronic carriers. J Med Virol. 2006;78(1):24-36.
16. Bracco C, Gallarate M, Badinella Martini M, Magnino C, D'Agnano S, Canta R, et al. Epidemiology, therapy and outcome of hepatocellular carcinoma between 2010 and 2019 in Piedmont, Italy. World J Gastrointest Oncol. 2024;16(3):761-72.
17. Tarao K, Nozaki A, Ikeda T, Sato A, Komatsu H, Komatsu T, et al. Real impact of liver cirrhosis on the development of hepatocellular carcinoma in various liver diseases-meta-analytic assessment. Cancer Med. 2019;8(3):1054-65.
18. Choi YM, Lee SY, Kim BJ. Naturally occurring hepatitis B virus reverse transcriptase mutations related to potential antiviral drug resistance and liver disease progression. World J Gastroenterol. 2018;24(16):1708-24.
19. Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, et al. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. Gut. 2001;48(2):251-9.
20. Kırdar S, Yaşa MH, Sayan M, Aydın N. HBV pol/S gene mutations in chronic hepatitis B patients receiving nucleoside/nucleotide analogues treatment. Mikrobiyol Bul. 2019;53(2):144-55. Turkish.
21. Yeh CT, Shen CH, Tai DI, Chu CM, Liaw YF. Identification and characterization of a prevalent hepatitis B virus X protein mutant in Taiwanese patients with hepatocellular carcinoma. Oncogene. 2000;19(46):5213-20.
22. Jun CH, Hong HJ, Chung MW, Park SY, Cho SB, Park CH, et al. Risk factors for hepatocellular carcinoma in patients with drug-resistant chronic hepatitis B. World J Gastroenterol. 2013;19(40):6834-41.
23. Bian CR, Li JJ, Song YW, Song LJ, Zhao J, Dong RM, et al. Analysis of characteristics of drug resistance gene mutation in HBV RT region of hepatitis B infected patients. Zhonghua Yu Fang Yi Xue Za Zhi. 2023;57(6):868-76. Chinese.
24. Zhang ZH, Wu CC, Chen XW, Li X, Li J, Lu MJ. Genetic variations of hepatitis B virus and its significance for pathogenesis. World J Gastroenterol. 2016;22(1):126-44.