

Hepatitis B virus genotypes and antiviral drug resistance mutations in treatment-naïve patients with chronic hepatitis B in Bacninh, Vietnam: a cross-sectional study

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Abstract

Background: Vietnam has one of the highest hepatitis B virus (HBV) infection rates, with approximately 8 million people affected. Although antiviral drug resistance mutations have been reported in treatment-naïve patients with chronic hepatitis B, there is limited data on primary drug resistance mutations in circulating genotypes within this population.

Objectives: This study aimed to investigate primary antiviral drug resistance mutations and common HBV genotypes in treatment-naïve patients with chronic hepatitis B, particularly in cases without well-characterized resistance profiles.

Design: A cross-sectional study.

Methods: We analyzed HBV genotypes and antiviral drug resistance mutations in 113 treatment-naïve patients with chronic hepatitis B in the Yenphong Medical Center, Bacninh Vietnam. The reverse transcriptase (RT) region of the HBV polymerase genes was sequenced to detect mutations.

Results: Genotypes B, C, and G were identified in 85.0% (96/133), 14.1% (16/133), and 0.9% (1/133) of treatment-naïve patients with chronic hepatitis B, respectively. Mutations in the RT region associated with antiviral drug resistance were detected in 32.7% (37/113) of patients. In addition, the most frequent resistance mutations were rtV207M (89.2%, 33/37), followed by A194T, L180M + M204V, V173L + M204I + L80I, and A181T + V207M + A181T, each observed in 2.7% (1/37). Notably, no significant associations were found between resistance mutations and HBV genotype, gender, age, hepatitis B e-antigen status, baseline HBV DNA levels, or level of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase.

Conclusion: This study highlights the presence of primary resistance mutations in treatment-naïve patients and underscores the importance of genotypic screening prior to initiating therapy. These findings may inform treatment strategies and help reduce the risk of treatment failure, liver cirrhosis, and hepatocellular carcinoma.

Keywords: antiviral drug resistance, Bacninh, chronic hepatitis B, hepatitis B virus (HBV), resistance mutations, Vietnam

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Introduction

Chronic hepatitis B virus (HBV) infection is a leading cause of liver decompensation, cirrhosis, hepatocellular carcinoma, and mortality worldwide, affecting an estimated 360 million

individuals and accounting for approximately 1 million deaths annually.^{1–3} According to the World Health Organization, the global prevalence of HBV infection varies substantially, with the highest rates reported in Africa and the Western

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Pacific.³ Vietnam, as a developing country in the Western Pacific region, has among the highest national HBV prevalence rates, with approximately 8 million individuals infected.⁴ The distribution of HBV in Vietnam is highly heterogeneous due to variations in ethnicity, socioeconomic status, and regional healthcare access.^{5,6}

Early detection, monitoring, and timely treatment of chronic hepatitis B infection are critical to reducing disease progression and mortality.⁷ Significant advances have been made in the treatment of chronic hepatitis B, particularly through the use of interferon-alpha and nucleotide analogs (NAs). Currently, approved NAs include six antiviral drugs: Lamivudine (LAM), Adefovir dipivoxil (ADV), Tenofovir disoproxil fumarate (TDF), Entecavir (ETV), Tenofovir alafenamide (TAF), and Telbivudine (LDT).^{8–10} Despite their efficacy, NAs can cause adverse effects that complicate clinical management. Moreover, long-term use may exert selective pressure, allowing drug-resistant HBV variants to emerge and persist.¹¹ Notably, treatment with NAs has been shown to induce specific resistance mutations, including in treatment-naïve patients with chronic hepatitis B.^{12,13} These resistance mutations typically occur in the reverse transcriptase (RT) region of the HBV polymerase gene. Initial investigations of such mutations focused on patients receiving LAM, ADV, ETV, and LDT.^{14–17} Among them, LAM-resistant mutations are the most frequently reported, with the rtM204I/V mutation being the most prevalent.¹⁸

The HBV genome is genetically diverse and has been classified into ten genotypes (A–J) based on a nucleotide divergence of $\geq 8\%$ across the entire genome. In addition, sub-genotypes differ from one another by at least 4%. The distribution of HBV genotypes varies significantly across racial groups and geographical regions. Genotype A is primarily found in Europe, where subgenotype A2 is predominant, and in Africa, where subgenotype A1 is more common. Other genotypes are distributed as follows: genotypes B and C in Asia; genotype D in Africa, Europe, the Mediterranean region, and India; genotype E in West and Southern Africa; genotype F in South and Central America; genotype G in France and the United States; genotype H in Mexico and Central America; genotype I in Vietnam and Laos; and the recently identified genotype J in the Ryukyu Islands of Japan.^{19,20} In Vietnam, genotypes B

and C are most prevalent, with genotype B being more common than genotype C.²¹ Conversely, genotypes A, I, and G are rarely observed, while sub-genotypes B2–B5 and C5–C16 are predominant.²¹ Given this distribution, the present study aimed to investigate primary antiviral drug resistance mutations in circulating HBV genotypes among treatment-naïve patients with chronic hepatitis B in Bacninh, Vietnam. The study also sought to evaluate the clinical relevance of these genotypes in guiding treatment strategies.

Materials and methods

Patients

This study included 113 treatment-naïve patients diagnosed with chronic hepatitis B between 2022 and 2023 at the Yenphong Medical Center in Bacninh, Vietnam. Inclusion criteria were as follows: (1) patients with an HBV viral load $\geq 10^3$ copies/mL at the time of initial sampling during the study period; (2) no prior treatment with HBV antiviral drugs; (3) complete laboratory results for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and hepatitis B e-antigen (HBeAg) rapid test; (4) age 18 years or older. Exclusion criteria included: (1) co-infection with hepatitis C virus (HCV) and/or human immunodeficiency virus (HIV); and (2) diagnosis of hepatocellular carcinoma. All enrolled patients had tested positive for hepatitis B surface antigen (HBsAg) for at least 6 months and were confirmed negative for both HIV and HCV. In addition, each patient had an HBV viral load $\geq 10^3$ copies/mL and had not received any prior antiviral treatment. The study was approved by the Ethics Committee of the Hanoi University of Public Health (Approval No. 224/2023/YTCC-HD3), dated May 10, 2023, granted by the director of the Yenphong Medical Center, dated March 28, 2023. This study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants. In cases where patients were unable to provide consent due to medical conditions, cognitive impairments, or severe illness, consent was obtained from their next of kin. Following ethics approval on May 10, 2023, patients recruited prior to this date, specifically those who had visited the Yenphong Medical Center in 2022, were included using stored serum samples. These patients were subsequently contacted by phone or during in-person follow-ups at

the medical center to obtain informed consent and additional clinical information. Especially, data including HBV viral load, treatment status, liver enzyme levels (ALT, AST, and GGT), HBeAg status, and age were retrieved from medical records.

Quantification of HBV

HBV DNA was detected and quantified using quantitative polymerase chain reaction (qPCR), in accordance with the manufacturer's instructions for the TOPSENSI® HBV QPCR Kit (ABT Equipment Co., Ltd., Hanoi, Vietnam). All tests were performed automatically at the Yenphong Medical Center, Bacninh, Vietnam.

Polymerase chain reaction

HBV DNA was extracted from 200 µL of serum using the DNA/RNAprep MAGBEAD kit (Nam Khoa Trading and Services Co., Ltd., Ho Chi Minh city, Vietnam), following the manufacturer's protocol. The RT region of the HBV gene (540 bp) was amplified via nested PCR using two sets of specific primers: CLC 882 for the first-round PCR, and CLC 188m for the second-round PCR. Amplifications were performed on a SimpliAmp Thermo Cycler (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The thermocycling protocol included an initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 57°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min.

Sequencing the HBV-RT region

The size of the PCR products was assessed by electrophoresis on a 2% agarose gel using a PowerPac Universal Bio-Rad electrophoresis machine (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR products were purified using the Illustra ExoStar Enzymatic cleanup system (Cytiva, Marlborough, MA, USA). Sequencing reactions were then performed on the target gene segment using a single primer (CLC 887 and CLC 188m) and dideoxynucleotide (ddNTP) labeled with fluorescent dyes: green for adenine, red for thymine, black for guanine, and blue for cytosine. Capillary electrophoresis was conducted on the ABI 3130xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturer's protocols. The resulting sequences were analyzed using Sequencing Analyzer

software, version 5.2 (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Sequence analysis

To confirm the specificity of each amplicon, the obtained sequences were analyzed using the GenBank Basic Local Alignment Search Tool (BLAST). Sequences from the same patient were aligned to a reference sequence, and any discrepancies were manually corrected through visual inspection using Sequencing Analyzer software, version 5.2 (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The consensus sequence of each HBV isolate was then submitted to <https://blast.ncbi.nlm.nih.gov/Blast.cgi> to determine the HBV subgenotype and to predict phenotypic resistance based on genotype-specific mutations in the polymerase gene (RT mutations).

Statistical analysis

All statistical tests were 2-tailed, and a *p*-value of <0.05 was considered the threshold for statistical significance. Data management and statistical analyses were performed using SPSS software, version 26.0 (IBM Corp, Armonk, NY, USA).

Results

Clinical characteristics of treatment-naïve patients with chronic hepatitis B

A total of 113 treatment-naïve patients with chronic hepatitis B were enrolled in this cross-sectional study at the Yenphong Medical Center, Bacninh, Vietnam. The clinical characteristics of the study population are presented in Table 1. Among these patients, 53.1% were male and 46.9% were female, with a mean age of 36 years (range: 18–71 years). Elevated levels of serum liver enzymes were observed in the following proportions of patients: ALT ≥ 41 U/L in 49.6%, AST ≥ 37 U/L in 41.6%, and GGT ≥ 50 U/L in 26.5%, with corresponding mean values of 59.4, 51.9, and 56.3 U/L, respectively. The mean serum HBV DNA level was 5.82 log₁₀ copies/mL (range: 2.8–8.98), and 39.8% of these patients were HBeAg seronegative. The distribution of HBV genotypes was as follows: genotype B in 96 patients (85%), genotype C in 16 patients (14.1%), and genotype G in one patient (0.9%).

Table 1. Clinical characteristics of treatment-naïve patients with chronic hepatitis B at the Yen Phong Medical Center, Bacninh, Vietnam from 2022 to 2023 ($n = 113$).

Characteristics	Treatment-naïve patients with chronic hepatitis B ($n = 113$)
Age, min–max (mean $\pm$ SD)	18–71 (36.0 $\pm$ 12.3)
Gender, n (%)	
Male	60 (53.1)
Female	53 (46.9)
ALT U/L, min–max (mean $\pm$ SD)	10–385 (59.4 $\pm$ 60.5)
Reference value of ALT ≥ 41 U/L, n (%)	56 (49.6)
AST U/L, min–max (mean $\pm$ SD)	11–281 (51.9 $\pm$ 51.1)
Reference value of AST ≥ 37 U/L, n (%)	47 (41.6)
GGT U/L, min–max (mean $\pm$ SD)	3.4–686 (56.3 $\pm$ 86.4)
Reference value of GGT ≥ 50 U/L, n (%)	30 (26.5)
HBeAg status, n (%)	
Positive	68 (60.2)
Negative	45 (39.8)
HBV DNA $\log_{10} \geq 4$, n (%)	90 (79.6)
HBV DNA level, min–max (mean $\pm$ SD)	2.8–8.98 (5.82 $\pm$ 1.84)
HBV genotype, n (%)	
B	96 (85)
C	16 (14.1)
G	1 (0.9)
Resistance mutation to antiviral drug, n (%)	37 (32.7)
ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma-glutamyl transferase; HBeAg, Hepatitis B e antigen; HBV, Hepatitis B virus.	

Mutations associated with antiviral drug resistance were identified in 37 patients (32.7%).

Mutation analysis in the HBV-RT region of treatment-naïve patients with chronic hepatitis B
Among the 117 treatment-naïve patients with chronic hepatitis B at the Yenphong Medical

Center, Bacninh, Vietnam, a total of 37 (32.7%) exhibited mutations in the RT region of the HBV polymerase gene that may be associated with resistance to antiviral drugs such as LAM, TDF, and/or ADV (Table 2). Mutations associated with LAM resistance were identified in 36 of the 37 samples. In addition, mutations linked to resistance to ADV and TDF were detected in two and one samples, respectively. The identified resistance-associated mutations included rtV207M, rtV173L + rtM204I + rtL80I, rtA181T + rtV207M + rtA181T, rtA194T and rtL180M + rtM204V (Table 2). Among these, the most frequent mutation was rtV207M, observed in 33 of the 37 samples, all of which were associated with LAM resistance. The combined rtL180M + rtM204V mutations were found in one of the 37 samples. Notably, the rtA194T mutation, detected in one sample, was associated with resistance to TDF but remained sensitive to LAM. Furthermore, two combined mutation patterns: rtV173L + rtM204I + rtL80I and rtA181T + rtV207M + rtA181T conferred resistance to both LAM and ADV, each identified in one of the 37 samples (Table 2).

Association between mutations and epidemiological characteristics of treatment-naïve patients with chronic hepatitis B

The association between resistance mutations and epidemiological characteristics, including HBV genotype, gender, age, HBeAg status, baseline HBV DNA levels, and serum levels of ALT, AST, and GGT, was not statistically significant among the 113 treatment-naïve patients with chronic hepatitis B ($p > 0.05$) (Table 3).

Discussion

Antiviral drug-resistant mutations have been reported even in treated treatment-naïve patients with chronic hepatitis B, and they carry important clinical implications. Monitoring both primary and secondary resistance mutations in treatment-naïve individuals is essential for determining the most effective therapeutic strategies and for preventing progression to cirrhosis and hepatocellular carcinoma.^{13,22} In this study, we analyzed the distribution of HBV genotypes and the presence of primary resistance mutations in 113 treatment-naïve patients with chronic hepatitis B at the Yenphong Medical Center, Bacninh, Vietnam. Three HBV genotypes were identified: B, C, and

Table 2. Virological and resistance mutation profile of treatment-naïve patients with chronic hepatitis B to antiviral drug ($n=37$).

Patient	Age (years)	Gender	Viral load ($\log_{10}$ UI/mL)	HBV DNA levels (copies/mL)	Genotype	Mutation rtHBV	HBeAg status	Resistance profile		
								LAM	ADV	TDF
173926	20	M	7.33	1.24×10^8	B	V207M	Positive	R		
188127	21	F	6.16	8.50×10^6	B	V207M	Positive	R		
192497	21	F	7.55	2.08×10^8	B	V207M	Positive	R		
205247	23	F	7.05	6.48×10^7	B	V207M	Positive	R		
007537	38	F	8.02	6.15×10^8	B	V207M	Positive	R		
007673	33	F	6.69	2.84×10^7	B	V207M	Negative	R		
012292	21	F	6.36	1.34×10^7	B	V207M	Positive	R		
017037	32	M	7.10	7.30×10^7	B	V207M	Positive	R		
019973	34	F	7.61	2.35×10^8	B	V207M	Positive	R		
025617	33	F	6.52	1.93×10^7	B	V207M	Positive	R		
029843	46	M	7.91	4.73×10^8	B	V207M	Negative	R		
031500	25	M	8.51	1.87×10^9	B	V207M	Positive	R		
031636	34	M	3.33	1.25×10^4	B	V207M	Negative	R		
034613	38	F	8.45	1.65×10^9	B	V207M	Negative	R		
081696	44	M	2.85	4.12×10^3	B	V207M	Positive	R		
036435	42	M	5.01	6.00×10^5	B	V207M	Positive	R		
037869	25	F	6.89	4.54×10^7	B	V207M	Positive	R		
041670	39	M	7.50	1.83×10^8	B	V173L + M204I + L80I	Negative	R	R	
041894	18	M	6.98	5.55×10^7	B	V207M	Positive	R		
043144	19	M	8.89	4.52×10^9	B	V207M	Positive	R		
048153	60	M	4.15	8.30×10^4	B	V207M	Negative	R		
048650	55	M	3.31	1.18×10^4	B	V207M	Negative	R		
048727	35	F	4.83	3.90×10^6	B	V207M	Positive	R		
060109	63	M	4.90	4.62×10^5	B	A181T + V207M + A181T	Negative	R	R	
060183	24	F	5.55	2.08×10^6	B	V207M	Positive	R		
060211	35	F	4.47	1.73×10^5	B	V207M	Positive	R		

(Continued)

Table 2. (Continued)

Patient	Age (years)	Gender	Viral load (log ₁₀ UI/mL)	HBV DNA levels (copies/mL)	Genotype	Mutation rtHBV	HBeAg status	Resistance profile		
								LAM	ADV	TDF
060335	36	F	3.73	3.13×10^4	B	V207M	Positive	R		
065705	54	M	3.44	1.59×10^4	B	A194T	Negative	S		R
065503	31	F	4.50	1.85×10^5	B	V207M	Negative	R		
065749	39	M	4.77	3.45×10^5	B	V207M	Negative	R		
065845	23	M	7.02	6.06×10^7	B	V207M	Positive	R		
067666	56	F	8.45	1.63×10^9	B	V207M	Negative	R		
076956	42	M	4.32	1.21×10^5	B	L180M + M204V	Negative	R		
080425	32	F	7.29	1.13×10^8	B	V207M	Positive	R		
080823	41	M	3.16	8.47×10^3	B	V207M	Negative	R		
081292	35	F	4.45	1.65×10^5	B	V207M	Positive	R		
081481	52	M	7.57	2.14×10^8	B	V207M	Positive	R		

ADV, adefovir dipivoxil; HBeAg, Hepatitis B e antigen; HBV, Hepatitis B virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate.

G. Resistance to LAM was common among these patients, with genotype B showing the highest prevalence of the rtV207M mutation.

The predominant HBV genotypes observed in Vietnam are B and C, while genotypes A, I, and G are relatively rare. Sub-genotypes commonly found include B2–B5 and C5–C16.²¹ Genotyping plays a crucial role in evaluating patient prognosis, diagnosis, and treatment, as the HBV transcription process has a high error rate, resulting in the emergence of numerous viral variants. Notably, Liu et al. demonstrated that the location of the mutation is genotype-dependent.²³ In this study, HBV genotypes were determined in all 113 treatment-naïve patients with chronic hepatitis B. Among these, genotype B accounted for 85% of cases, genotype C for 14.1%, and genotype G for 0.9%. Although genotype G was rarely detected, genotypes B and C remain the most epidemiologically and clinically significant in Vietnam. Previous studies have shown that genotypes B and C are commonly associated with vertical transmission.²⁴ In addition, Lin et al. reported that genotype C is associated with a higher risk of cirrhosis and hepatocellular carcinoma compared

to other genotypes.¹⁹ For patients infected with genotypes B and C who have not previously received antiviral treatment with ETV or TDF is recommended that treatment is indicated.²⁵ However, treatment decisions should be made with caution, as HBV genotype may influence the frequency and pattern of treatment-emergent resistance mutations in treatment-naïve patients.

Further investigations into the presence of antiviral drug resistance mutations in treatment-naïve patients with chronic hepatitis B are warranted due to its significant clinical implications. In this study, direct sequencing of HBV DNA targeting the RT region of the polymerase gene was conducted to monitor resistance-associated mutations. Resistance mutations were identified in 37 of the 113 treatment-naïve patients (32.7%). These findings are consistent with previous reports, which have documented RT mutations in treatment-naïve patients at the rate of 56%,²⁶ 38%,²⁷ 46%,²⁸ and 47.9%.²⁹ However, in contrast, a study conducted in the Northern and Northeastern regions of Brazil found that only 2.6% (5/189) of treatment-naïve patients harbored primary resistance-associated mutations.³⁰

Table 3. Association of epidemiological characteristics of patients with resistance mutations and without resistance mutations in the HBV-RT region.

Characteristics		Group with mutation (<i>n</i> = 37)	Group without mutation (<i>n</i> = 76)	<i>p</i> -Value
HBV genotype, <i>n</i> (%)	B	37 (100)	59 (77.6)	> 0.05
	C		16 (21.1)	
	G		1 (1.3)	
Gender, <i>n</i> (%)	Male	19 (51.4)	41 (53.9)	>0.05
	Female	18 (48.6)	35 (46.1)	
HBeAg status, <i>n</i> (%)	Positive	23 (62.2)	45 (59.2)	>0.05
	Negative	14 (37.8)	31 (40.8)	
Age, <i>n</i> (%)	≥40 years old	11 (29.7)	22 (28.9)	>0.05
	<40 years old	26 (70.3)	54 (71.1)	
HBV DNA, log ₁₀ <i>n</i> (%)	≥4	31 (83.8)	59 (77.6)	>0.05
	<4	6 (16.2)	17 (22.4)	
ALT, <i>n</i> (%)	≥41 U/L	16 (42.2)	40 (52.6)	>0.05
	<41 U/L	21 (57.8)	36 (47.4)	
AST, <i>n</i> (%)	≥37 U/L	13 (35.1)	34 (44.7)	>0.05
	<37 U/L	24 (64.9)	42 (55.3)	
GGT, <i>n</i> (%)	≥50 U/L	9 (24.3)	23 (30.3)	>0.05
	<50 U/L	27 (75.7)	53 (69.7)	
ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate.				

The variation in mutation prevalence among studies may be attributed to factors such as sample size, geographic region, and patient lifestyle. In Asia, the reported prevalence of resistance mutations among treatment-naïve patients ranges from 1% to 20%.³⁰ In Vietnam, where chronic HBV infection remains highly endemic, approximately 6% of the adult population are carriers, a situation influenced by the country's tropical climate and developing status. This presents ongoing challenges to public health and social infrastructure.

Based on the 42 known RT mutation sites and the classification system proposed by Liu *et al.*,²³ the mutations identified in this study were

primarily categorized as primary drug resistance mutations, those that directly confer resistance by reducing drug susceptibility. These included amino acid substitutions such as A194T, A181T, M204I, and M204V. The secondary or compensatory mutations, which compensate for the impaired replication fitness defects caused by primary mutations, included L80I, L180M, and V173L. In addition, the V207M mutation, while associated with drug resistance, is currently considered a putative resistance mutation, as its role has yet to be validated through *in vitro* studies.

The rtA194T mutation may be associated with resistance to TDF. *In vitro* studies have shown that this mutation reduces sensitivity to TDF,

particularly when it co-occurs with LAM-resistant mutations such as rtM204V and rtL180M. However, the clinical significance of the rtA194T mutation remains unclear and warrants further investigation through long-term follow-up studies.^{16,31} HBeAg-negative patients may be at greater risk of developing the rtA194T mutation and, consequently, resistance to antiviral therapy. In such cases, ETV may represent a more suitable option.³² Among the mutations associated with LAM resistance, rtL180M + rtM204V and rtV207M are particularly notable. The rtM204V mutation is considered a primary resistance mutation due to its direct effect on reducing HBV susceptibility to LAM. In contrast, rtL180M is classified as a compensatory or secondary mutation, as it may restore or enhance the replication capacity of the virus impaired by primary mutations.^{33,34} The rtM204V mutation is one of the most frequently observed in studies of treatment-naïve patients.³⁵ Along with the V207M mutation, it is strongly associated with LAM resistance. Nevertheless, according to current guidelines for the diagnosis and treatment of chronic hepatitis B, LAM is no longer recommended as a first-line antiviral agent due to a low genetic barrier to resistance.²⁵ The rate of antiviral drug resistance with LAM can reach approximately 80% after 5 years of therapy.^{36,37} In accordance with recommendations from the Vietnamese Ministry of Health, ETV and TDF are the preferred first-line agents for chronic hepatitis B who have not yet developed cirrhosis. While resistance mutations to ADV are more commonly observed in patients previously treated with LAM, they are relatively rare in treatment-naïve individuals.³⁸ Previous studies have identified seven key mutations associated with ADV resistance: rtL80V/I,³⁹ rtS85A,^{40,41} rtV84M,⁴¹ rtA181V/T,^{41,42} rtV214A,^{40,41} rtQ215S/H,^{40,41} and rtN236T.^{41,42} In contrast, findings from the present study revealed the presence of the rtL80I and rtA181T mutations in treatment-naïve patients with chronic hepatitis B.

Patients with antiviral drug resistance mutations were compared with those without mutations, and no significant differences were observed. These findings are consistent with those of the previous study.³⁰ However, Zhang et al. conducted a meta-analysis to evaluate the prevalence of naturally occurring drug resistance mutations in treatment-naïve patients with chronic hepatitis B.⁴³ Their analysis demonstrated that male patients, individuals infected with HBV genotype C, and those

who were HBeAg-negative exhibited a higher rate of natural mutations compared to female patients, those with HBV genotypes B or D, and HBeAg-positive individuals.⁴³ In addition, prior studies have shown that serum levels of ALT, AST, and GGT are not significantly associated with the presence of antiviral resistance mutations.⁴³ In Vietnam, only a limited number of studies have investigated drug resistance mutations in treatment-naïve patients.^{5,23} Therefore, further research involving larger cohorts of treatment-naïve patients with chronic hepatitis B is warranted to better understand the evolving patterns and clinical relevance of resistance mutations.

In conclusion, genotypic monitoring is essential for identifying antiviral drug resistance mutations in treatment-naïve patients with chronic hepatitis B, as preexisting mutations in the RT region of the polymerase gene are associated with reduced drug susceptibility. The therapeutic efficacy of antiviral agents may be compromised by various factors, including infection with drug-resistant HBV variants. Consequently, antiviral treatment may not always be effective, even in patients who have not previously received therapy. Implementing HBV genotyping prior to treatment initiation may aid clinical decision making, minimize treatment failure, and reduce the risk of disease progression to cirrhosis or hepatocellular carcinoma.

Declarations

Ethics approval and consent to participate

All participants signed an informed consent form, and next of kin provided consent only in cases where participants were deemed incapable of providing full informed consent due to medical conditions, cognitive impairments, or severe illness. The study and the consent procedures were approved by the Ethics Committee of the Hanoi University of Public Health (Approval No. 224/2023/YTCC-HD3), dated May 10, 2023.

Consent for publication

Not applicable.

Author contributions

Minh-Cong Hoang: Conceptualization; Data curation; Formal analysis; Investigation; Project administration; Writing – original draft; Writing – review & editing.

Hong-Quan Duong: Conceptualization; Investigation; Methodology; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Van-Duyet Le: Formal analysis; Investigation; Writing – original draft; Writing – review & editing.

Thi-Thuy-Nga Nguyen: Data curation; Formal analysis; Investigation; Writing – original draft.

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References

- GBD 2019 Hepatitis B Collaborators. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Lancet Gastroenterol Hepatol* 2022; 7(9): 796–829.
- Polaris Observatory Collaborators. The global prevalence, a cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modeling study. *Lancet Gastroenterol Hepatol* 2023; 8(10): 879–907.
- Mahamat G, Kenmoe S, Akazong EW, et al. Global prevalence of hepatitis B virus serological markers among healthcare workers: a systematic review and meta-analysis. *World J Hepatol* 2021; 13(9): 1190–1202.
- Flower B, Hong DD, Kim HVT, et al. Seroprevalence of Hepatitis B, C and D in Vietnam: a systematic review and meta-analysis. *Lancet Reg Health West Pac* 2022; 24: 100468.
- Le TDT, Nguyen TB, Huynh TXL, et al. Hepatitis B virus infection in southern Khmer people in Cau Ke district, Tra Vinh province in 2021. *Vietnam Med J* 2022; 511(1): 16–20.
- Pham NT, Nguyen TTT, Tran DP, et al. Prevalence and factors associated with chronic hepatitis B infection among adults in the Central Highlands, Vietnam. *AIMS Med Sci* 2020; 7(4): 337–346.
- Nguyen TK and Le DV. Resistant mutations within the hepatitis B virus reverse transcriptase sequence in treatment failure patients with chronic HBV infection in Vietnam. *J Glob Antimicrob Resist* 2023; 33: 35–41.
- Zhang X, Chen X, Wei M, et al. Potential resistant mutations within HBV reverse transcriptase sequences in nucleos(t)ide analogues experienced patients with hepatitis B virus infection. *Sci Rep* 2019; 9(1): 8078.
- Leowattana W and Leowattana T. Chronic hepatitis B: new potential therapeutic drugs target. *World J Virol* 2022; 11(1): 57–72.
- European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; 67(2): 370–398.
- Sheldon J and Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 2008; 61(4): 766–768.
- Jardi R, Rodriguez-Frias F, Schaper M, et al. Hepatitis B virus polymerase variants associated with entecavir drug resistance in treatment-naïve patients. *J Viral Hepat* 2007; 14(12): 835–840.
- Nguyen MH, Garcia RT, Trinh HN, et al. Prevalence of hepatitis B virus DNA polymerase mutations in treatment-naïve patients with chronic hepatitis B. *Aliment Pharmacol Ther* 2009; 30(11–12): 1150–1158.
- Selabe SG, Lukhwareni A, Song E, et al. Mutations associated with lamivudine-resistance in therapy-naïve hepatitis B virus (HBV) infected

- patients with and without HIV co-infection: implications for antiretroviral therapy in HBV and HIV co-infected South African patients. *J Med Virol* 2007; 79(11): 1650–1654.
15. Rodriguez C, Chevaliez S, Bensadoun P, et al. Characterization of the dynamics of hepatitis B virus resistance to adefovir by ultra-deep pyrosequencing. *Hepatology* 2013; 58(3): 890–901.
16. Tenney DJ, Rose RE, Baldick CJ, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; 49(5): 1503–1514.
17. Zhang Y, Lian JQ, Li Y, et al. Telbivudine plus adefovir therapy for chronic hepatitis B patients with virological breakthrough or genotypic resistance to telbivudine. *Eur J Gastroenterol Hepatol* 2013; 25(7): 814–819.
18. Leung N. Treatment of chronic hepatitis B: case selection and duration of therapy. *J Gastroenterol Hepatol* 2002; 17(4): 409–414.
19. Lin CL and Kao JH. Natural history of acute and chronic hepatitis B: the role of HBV genotypes and mutations. *Best Pract Res Clin Gastroenterol* 2017; 31(3): 249–255.
20. Tatematsu K, Tanaka Y, Kurbanov F, et al. A genetic variant of hepatitis B Virus Divergent from Known Human and Ape Genotypes Isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; 83(20): 10538–10547.
21. Ho PT, Balzanelli MG, Distratis P, et al. Characteristics of hepatitis B virus genotype and sub-genotype in hepatocellular cancer patients in Vietnam. *Diagnostics (Basel)* 2022; 12(10): 2392.
22. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295(1): 65–73.
23. Liu BM, Li T, Xu J, et al. Characterization of potential antiviral resistance mutations in hepatitis B virus reverse transcriptase sequences in treatment-naïve Chinese patients. *Antiviral Res* 2010; 85(3): 512–519.
24. Kramvis A and Kew MC. Molecular characterization of subgenotype A1 (subgroup Aa) of hepatitis B virus. *Hepatol Res* 2007; 37(s1): S27–S32.
25. Hou J, Wang G, Wang F, et al. Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update). *J Clin Transl Hepatol* 2017; 5(4): 297–318.
26. Lao DT, Truong KP, Mai NL, et al. Application of realtime PCR technique to determine genotype, amount of virus in blood and resistance characteristics medicine to treat hepatitis B virus in patients at Dong Thap General Hospital. *Ho Chi Minh City Open University J Science* 2014; 9(1): 63–73.
27. Aberle SW, Kletzmayer J, Watschinger B, et al. Comparison of sequence analysis and the INNO-LiPA HBV DR line probe assay for detection of Lamivudine-resistant hepatitis B virus strains in patients under various clinical conditions. *J Clin Microbiol* 2001; 39(5): 1972–1974.
28. Pollicino T, Isgro G, Di Stefano R, et al. Variability of reverse transcriptase and overlapping S gene in hepatitis B virus isolates from untreated and lamivudine-resistant chronic hepatitis B patients. *Antivir Ther* 2009; 14(5): 649–654.
29. Singla B, Chakraborti A, Sharma BK, et al. Hepatitis B virus reverse transcriptase mutations in treatment Naïve chronic hepatitis B patients. *J Med Virol* 2013; 85(7): 1155–1162.
30. Pacheco SR, Dos Santos MIMA, Stocker A, et al. Genotyping of HBV and tracking of resistance mutations in treatment-naïve patients with chronic hepatitis B. *Infect Drug Resist* 2017; 10: 201–207.
31. Funk ML, Rosenberg DM and Lok ASF. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. *J Viral Hepat* 2002; 9(1): 52–61.
32. Amini-Bavil-Olyae S, Herbers U, Sheldon J, et al. The rtA194T polymerase mutation impacts viral replication and susceptibility to tenofovir in hepatitis B e antigen-positive and hepatitis B e antigen-negative hepatitis B virus strains. *Hepatology* 2009; 49(4): 1158–1165.
33. Hoofnagle JH, Doo E, Liang TJ, et al. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; 45(4): 1056–1075.
34. Zoulim F and Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009; 137(5): 1593–1608.e1-2.
35. Fung J, Lai CL, Fong DYT, et al. Correlation of liver biochemistry with liver stiffness in chronic hepatitis B and development of a predictive model for liver fibrosis. *Liver Int* 2008; 28(10): 1408–1416.
36. Lok ASF and McMahon BJ. Chronic hepatitis B: updated 2009. *Hepatology* 2009; 50(3): 661–662.

37. Shaw T, Bartholomeusz A and Locarnini S. HBV drug resistance: mechanisms, detection and interpretation. *J Hepatol* 2006; 44(3): 593–606.
38. Kwon HC, Cheong JY, Cho SW, et al. Emergence of adefovir-resistant mutants after reversion to YMDD wild-type in lamivudine-resistant patients receiving adefovir monotherapy. *J Gastroenterol Hepatol* 2009; 24(1): 49–54.
39. Lee YS, Chung YH, Kim JA, et al. Hepatitis B virus with the rtL80V/I mutation is associated with a poor response to adefovir dipivoxil therapy. *Liver Int* 2009; 29(4): 552–556.
40. Gomes-Gouvêa MS, Ferreira AC, Teixeira R, et al. HBV carrying drug-resistance mutations in chronically infected treatment-naïve patients. *Antivir Ther* 2015; 20(4): 387–395.
41. Ryu SH and Chung YH. Resistance to adefovir in patients with chronic hepatitis B. *Korean J Hepatol* 2006; 12(4): 484–492.
42. Lok AS, Zoulim F, Locarnini S, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; 46(1): 254–265.
43. Zhang Q, Liao Y, Cai B, et al. Incidence of natural resistance mutations in naïve chronic hepatitis B patients: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2015; 30(2): 252–261.

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