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Genotype-specific Prevalence of Precore and Core Promoter Mutations in HBeAg-negative Chronic Hepatitis B Patients in Iraq

Huda Salih Jassim,[1] Mothana Ali Khali,[1,*] and Yasin H. Majeed[2]

[1] *Department of Microbiology, College of Medicine, University of Anbar, Ramadi, Anbar, Iraq.*
[2] *Department of Internal Medicine/Gastroenterology, College of Medicine, University of Anbar, Ramadi, Anbar, Iraq.*

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ABSTRACT

Background: The distribution of hepatitis B virus (HBV) genotypes and viral mutations significantly influences disease progression in HBeAg-negative chronic hepatitis B (CHB), particularly in endemic regions where molecular epidemiological data are limited.
Objectives: To assess the molecular epidemiology of HBV in Iraqi patients with HBeAg-negative chronic hepatitis B, focusing on genotype prevalence, key precore (G1896A, G1898A) and basal core promoter (A1762T, G1764A) mutations, and their prognostic significance for advanced hepatic disease.
Materials and methods: This cross-sectional study enrolled 150 HBeAg-negative CHB patients at Baghdad Teaching Hospital, Baghdad, Iraq, from January to April 2025. HBV genotyping and mutation analysis were performed using polymerase chain reaction-Sanger sequencing. Hepatic fibrosis was assessed using transient elastography, which employs established fibrosis staging (F0-F4) with specific cutoff values. Fibrosis stages were classified as: F0-F1 (<7.0 kPa), F2 (7.0-9.4 kPa), F3 (9.5-12.4 kPa), and F4 (≥12.5 kPa).
Results: Genotype D predominated (85.3%), exhibiting significantly elevated viral loads compared to non-D genotypes (4.9±1.3 *vs.* 4.3±1.0 log I. U/mL, P-value = 0.02). G1896A mutation prevalence reached 54.7% overall, with marked elevation in genotype D patients (61.7% *vs.* 20.0% in genotype C, P-value = 0.004). Advanced fibrosis (F3-F4) was observed in 36.7% of patients, with G1896A mutation (OR=3.1, 95% CI: 1.4-6.9) and BCP mutations (OR=4.3, 95% CI: 1.9-9.8) serving as independent predictors. Laboratory parameters, including albumin levels <3.5 g/dL and platelet counts $<150\times10^3/\mu$L, demonstrated strong associations with the development of cirrhosis.
Conclusion: HBV genotype D and associated precore mutations are significant predictors of severe clinical outcomes in Iraqi HBeAg-negative CHB patients, supporting integrated molecular diagnostics for enhanced risk stratification in resource-limited settings.
Keywords: Hepatitis B virus; Genotype D; Precore mutations; Basal core promoter; HBeAg-negative; Liver fibrosis.

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INTRODUCTION

Chronic hepatitis B (CHB) infection is a common disease worldwide with a high rate of infection (approximately 296 million persons) and fatality (annual deaths of 820000 persons) due to hepatic cirrhosis and hepatocellular carcinoma [1, 2]. There is a wide range of clinical manifestations from CHB infection due to host immune responses, environmental factors, and complex interactions between viral genetic factors. The disease progression and response to treatment depend on the hepatitis B virus (HBV) genotype and specific viral mutations [2, 3].

HBV has ten distinct genotypes (from A to J), which differ in the geographical distributions and clinical implications [4]. Genotype D is distributed mainly in the Mediterranean, Middle East, and northern Africa, and is associated with a high risk of hepatocellular carcinoma [5]. Although genotype D is predominant in Iraq, comprehensive studies regarding

* Corresponding author: E-mail: med.mu.707070@uoanbar.edu.iq

molecular and epidemiological variables remain scarce, necessitating future investigations to underscore local patterns of the disease.

HBeAg-negative CHB is a prevalent disease variant characterized by mutations in the precore and basal core promoter (BCP) regions that decrease production of HBeAg [5]. While the G1896A mutation prevents HBeAg synthesis, BCP mutations (A1762T/G1764A) downregulate HBeAg and may increase viral replication [6]. These mutations enhanced the risk of liver inflammation and accelerated fibrosis, as well as poor antiviral drug responses, particularly in genotype D HBV infections [6, 7].

Owing to the advancement in the molecular diagnostics tools, there is an improvement in the HBV genotyping and mutation diagnosis in the clinical setting [8]. However, the clinical efficacy and cost effectiveness necessitates examination in a certain population, specifically with constraints of the resources.

Iraq, as part of the Middle Eastern populations, has a unique epidemiological and genetic features that greatly affects the HBV pathogenesis and antiviral therapy responses [8]. CHB management in Iraq faces considerable challenges because of low or unavailable diagnostic tests, limited antiviral drugs, and greatly reduction of healthcare infrastructure due to political conflict and poor security condition. Additionally, there is a sustained HBV transmission risks through nosocomial infections, unsafe injection practices, and inadequate vaccination coverage [6].

It is of utmost importance to understand the HBV molecular characteristics in this population to provide appropriate antiviral therapy. Hence, this study aimed to determine the distribution of HBV genotype among HBeAg-negative CHB patients in Iraq, assess precore mutations (G1896A, G1898A) and BCP mutations (A1762T, G1764A), and examined their association with disease severity.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Gastroenterology and Hepatology, Baghdad Teaching Hospital, Iraq. The study covered a period from January to April 2025. The study was approved by the Ethical Approval Committee of the University of Anbar (Reference number 38). Informed consent was obtained from participants, and they had the right to withdraw from the current study at any time without any consequences. Additionally, the study was conducted according to the updated version (October 2024) of the Helsinki principles.

HBeAg-negative CHB was defined as persistent HBsAg positivity of more than six months, HBeAg negativity with anti-HBe positivity, and detectable HBV deoxyribonucleic acid (DNA) levels (>2000 IU/mL) [8]. Patients with an age of 18 years or older from both sexes diagnosed with HBeAg-negative CHB who had measurable HBV DNA levels and gave informed consent were enrolled in the present study. Patients who had other than HBV infections [(hepatitis C virus, hepatitis D virus, or human immunodeficiency virus (HIV)]; presented with severe hepatic disease or hepatocellular carcinoma; pregnant or breastfeeding women; had undergone organ transplantation; had any active tumor; demonstrated severe kidney dysfunction were excluded.

The sample size was calculated assuming a 60% prevalence of the G1896A mutation among genotype D patients, 8% precision, and a 95% confidence level [9]. Using the formula $n = Z^2p(1\text{-}p)/d^2$, the minimum required sample was 144, and 150 patients were enrolled to allow for attrition. Peripheral venous blood was collected in EDTA tubes and stored at -80 °C until analysis. HBV DNA was quantified using real-time (PCR) polymerase chain reaction (COBAS TaqMan HBV Test, Roche Diagnostics; detection range 20-1.7 × 10^8 IU/mL). HBV genotyping targeted the S gene, amplified by PCR using genotype D-specific primers (yielding a 322 bp amplicon), and sequenced by the Sanger method. PCR conditions included 35 cycles of denaturation (95 °C, 30 s), annealing (58 °C, 30 s), and extension (72 °C, 45 s). Phylogenetic analysis was conducted using MEGA X with GenBank reference sequences (AM282986 (D), AB246345 (A), AB033554 (C), X75657 (E)). Sequence alignment and analysis were completed with BioEdit v7.2.5.

Liver fibrosis assessment utilized transient elastography (FibroScan® 502 Touch, Echosens) performed by certified operators following standardized protocols. Measurements were considered reliable when ten valid acquisitions were obtained with a success rate >60% and an interquartile range/median ratio <30%. Fibrosis stages were classified as: F0-F1 (<7.0 kPa), F2 (7.0-9.4 kPa), F3 (9.5-12.4 kPa), and F4 (≥12.5 kPa).

Liver fibrosis was evaluated using transient elastography (FibroScan® 502 Touch, Echosens) by certified operators following standard protocols. Reliable measurements required at least ten valid readings, a success rate >60%, and an interquartile range/median ratio <30%. Fibrosis was staged as F0-F1 (<7.0 kPa, mild or none), F2 (7.0-9.4 kPa, moderate), F3 (9.5-12.4 kPa, severe), and F4 (≥12.5 kPa, cirrhosis). Advanced fibrosis was defined as stages F3-F4 [10].

Comprehensive clinical data were collected using standardized case report forms, including demographic characteristics, medical history, physical examination findings, and treatment details. Laboratory assessments encompassed complete blood count, comprehensive metabolic panel, liver function tests, coagulation profile, and alpha-fetoprotein levels.

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM, Armonk, NY, USA). Data normality was tested with the Shapiro-Wilk or Kolmogorov-Smirnov tests. Normally distributed data were presented as mean ± SD and compared using Student's t-test or one-way ANOVA, while non-normal data were shown as median (IQR) and analyzed using the Mann-Whitney U or Kruskal-Wallis tests. Categorical variables were expressed as frequencies and percentages and compared using the chi-square or Fisher's exact test. Multivariate logistic regression was used to identify predictors of advanced fibrosis and cirrhosis, with odds ratios (OR) and 95% confidence intervals (CI). A P-value < 0.05 was considered a statistically significant difference.

RESULTS

The mean age of our patients was 45.2±12.8 years, with male predominance (n=94, 62.7%) and the majority from urban residence (n=122, 81.3%). The mean duration of HBV infection was 8.7±4.3 years, with 68 (45.3%) of participants receiving antiviral therapy. Genotype D was detected in the majority of patients (n=128, 85.3%). Genotype D infections revealed significantly higher viral loads compared to non-genotype D infections (4.9±1.3 vs. 4.3±1.0 log IU/mL, P-value = 0.02) as illustrated in Table 1.

The results found that the G1896A mutation was detected

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Table 1. Demographic and clinical characteristics of 150 patients with hepatitis B virus infections according to its genotype distribution *.

Characteristic	Total (n=150)	Genotype D (n=128)	Non-genotype D (n=22)	P-value
Age (years), mean ± SD	45.2 ± 12.8	46.1 ± 13.2	43.3 ± 10.4	0.32
Male sex, n (%)	94 (62.7)	80 (62.5)	14 (63.6)	0.92
Urban residence, n (%)	122 (81.3)	105 (82.0)	17 (77.3)	0.59
BMI (kg/m^2), mean ± SD	26.8 ± 4.2	27.1 ± 4.3	25.4 ± 3.8	0.08
Diabetes mellitus, n (%)	38 (25.3)	33 (25.8)	5 (22.7)	0.76
Family history of HBV, n (%)	72 (48.0)	62 (48.4)	10 (45.5)	0.79
Viral load (log IU/mL), mean ± SD	4.8 ± 1.2	4.9 ± 1.3	4.3 ± 1.0	0.02*
ALT (U/L), mean ± SD	89.6 ± 36.2	91.4 ± 37.8	85.1 ± 32.1	0.41
Platelets ($\times 10^3/\mu$L), mean ± SD	198.3 ± 73.5	192.7 ± 75.2	210.5 ± 68.4	0.27

* P-value < 0.05 statistically significant difference. SD: Standard deviation, BMI: Body mass index, ALT: Alanine aminotransferase.

Table 2. Distribution of precore and basal core promoter mutations in 150 patients with hepatitis B virus infection according to its genotypes *.

Mutation	Overall (N=150)	Genotype D (N=128)	Genotype C (N=10)	Other genotypes (N=12)	P-value*
G1896A, n (%)	82 (54.7)	79 (61.7)	2 (20.0)	1 (8.3)	0.004
G1898A, n (%)	18 (12.0)	15 (11.7)	2 (20.0)	1 (8.3)	0.63
A1762T, n (%)	25 (16.7)	20 (15.6)	3 (30.0)	2 (16.7)	0.25
G1764A, n (%)	25 (16.7)	24 (18.8)	1 (10.0)	0 (0.0)	0.21
Combined A1762T/G1764A, n(%)	20 (13.3)	17 (13.3)	1 (10.0)	2 (16.7)	0.58

* P-value comparing genotype D vs. non-genotype D.

in 82 patients (54.7%), with significantly (P-value = 0.004) higher prevalence among genotype D patients (n=79, 61.7%) compared to genotype C patients (n=2, 20%). There were no significant associations (P-value > 0.05) between genotype D and other genotypes regarding other mutations, Table 2.

The majority of patients were with a hepatic fibrosis of F0-F1 (n=95, 63.3%). The prevalence of the G1896A mutation revealed a progressive increase with fibrosis stage (P-value for trend < 0.001) as shown in Figure 1.

Multivariate analysis found that the G1896A mutation

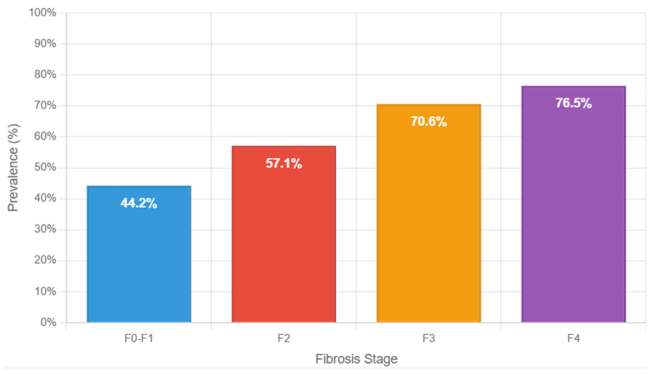

Figure 1. Relationship of the G1896A mutation and fibrosis stages [F0-F1 (<7.0 kPa, mild or none), F2 (7.0-9.4 kPa, moderate), F3 (9.5-12.4 kPa, severe), and F4 (≥12.5 kPa, cirrhosis). Advanced fibrosis was defined as stages F3-F4]. P-value for trend < 0.001.

independently predicted advanced (F3-F4) hepatic fibrosis (OR=3.1, 95% CI: 1.4-6.9, P-value = 0.005). Combined BCP mutations (A1762T/G1764A) revealed even stronger association with advanced (F3-F4) hepatic fibrosis (OR=4.3, 95% CI: 1.9-9.8, P-value = 0.001) as illustrated in Table 3.

Albumin levels decreased from 3.8±0.5 g/dL in F0-F1 to 2.6±0.4 g/dL in F4 (P-value < 0.001), while platelet counts declined from 228.4±64.2×10^3/μL to 112.9±32.8×10^3/μL (P-value ¡ 0.001). Multivariate analysis identified albumin <3.5 g/dL (OR=5.2, 95% CI: 2.1-12.9) and platelets <150×10^3/μL (OR=6.7, 95% CI: 2.8-16.1) as independent predictors of cirrhosis (Table 4).

Ascites developed in 22.0% of G1896A-positive patients compared to 10.3% of mutation-negative patients (relative risk=2.13, P-value = 0.05). Similarly, hepatomegaly was more prevalent in G1896A-positive patients (25.6% vs. 13.2%, P-value = 0.04), as were hypoalbuminemia (59.8% vs. 32.4%, P-value = 0.001) and thrombocytopenia (42.7% vs. 22.1%, P-value = 0.006), as indicated in Table 5.

The treatment response analysis at 12 months showed that virological response (HBV DNA < 2000 IU/mL) was achieved in 52.5% of G1896A-positive patients compared to 71.4% of mutation-negative patients (P-value = 0.045).

Fibro-Scan showed the highest diagnostic accuracy (AUROC 0.958, 95% CI: 0.923-0.993), followed by FIB-4 (AUROC 0.812) and APRI (AUROC 0.798), as shown in Table 6.

Based on the results of multivariate analysis, a composite risk score was developed that incorporates both virological and biochemical parameters. The score reported excellent discriminatory capacity, effectively stratifying patients into distinct risk categories as indicated in Table 7.

Table 3. Relationship between hepatitis B virus mutations and advanced fibrosis (F3-F4) in 150 patients*.

Mutation Status	F0-F2 (N=116)	F3-F4 (N=34)	Univariate OR (95% CI)	P-value	Adjusted OR (95% CI)†	P-value
G1896A present	53 (45.7)	29 (85.3)	3.4 (1.6–7.1)	0.001	3.1 (1.4–6.9)	0.005
G1898A present	14 (12.1)	4 (11.8)	1.0 (0.3–3.1)	0.96	0.9 (0.3–2.8)	0.85
A1762T present	15 (12.9)	10 (29.4)	3.2 (1.3–7.7)	0.009	2.8 (1.1–7.0)	0.03
G1764A present	16 (13.8)	9 (26.5)	2.6 (1.1–6.2)	0.03	2.3 (0.9–5.8)	0.07
A1762T/G1764A combined	12 (10.3)	8 (23.5)	3.0 (1.2–7.8)	0.02	4.3 (1.9–9.8)	0.001

* F3-F4: Advanced fibrosis (F3 = severe fibrosis 9.5-12.4 kPa; F4 = cirrhosis ≥12.5 kPa). Data were presented as n (%); †Adjusted for age, sex, viral load, and treatment status; OR: Odds Ratio; CI: Confidence Interval.

Table 4. Laboratory parameters by fibrosis stage and predictors of liver cirrhosis in 150 patients *.

Parameter	F0-F1 (N=95)	F2 (N=21)	F3 (N=17)	F4 (N=17)	P-value
ALT (U/L)	78.3 ± 29.1	95.2 ± 34.5	108.7 ± 38.2	121.6 ± 41.2	<0.001
AST (U/L)	65.2 ± 24.3	82.4 ± 28.9	96.3 ± 31.5	112.8 ± 35.7	<0.001
Albumin (g/dL)	3.8 ± 0.5	3.5 ± 0.5	3.0 ± 0.6	2.6 ± 0.4	<0.001
Platelets ($\times 10^3/\mu$L)	228.4 ± 64.2	186.5 ± 56.8	152.3 ± 48.2	112.9 ± 32.8	<0.001
INR	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.4 ± 0.3	<0.001
AFP (ng/mL)	52.1 ± 28.3	76.4 ± 35.8	95.2 ± 42.1	104.3 ± 37.9	<0.001

* Independent Predictors of Cirrhosis (Multivariate Analysis):
- Albumin <3.5 g/dL: OR=5.2 (95% CI: 2.1-12.9), P-value < 0.001.
- Platelets <$150\times10^3/\mu$L: OR=6.7 (95% CI: 2.8-16.1), P-value <0.00.
- Viral load >20,000 IU/mL: OR=3.1 (95% CI: 1.3-7.4), P-value = 0.01.
- G1896A + Genotype D: OR=2.8 (95% CI: 1.1-7.2), P-value = 0.03.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International normalized ratio; AFP: Alpha-fetoprotein.

Table 5. Relationship of clinical outcomes and complications according to G1896A mutation status in 150 patients*.

Clinical Outcome	G1896A positive (N=82)	G1896A negative (N=68)	Relative risk (95% CI)	P-value
Ascites, n (%)	18 (22.0)	7 (10.3)	2.13 (0.94–4.84)	0.05
Hepatomegaly, n (%)	21 (25.6)	9 (13.2)	1.94 (0.94–3.99)	0.04
Splenomegaly, n (%)	28 (34.1)	14 (20.6)	1.66 (0.94–2.92)	0.07
Albumin <3.5 g/dL, n (%)	49 (59.8)	22 (32.4)	1.85 (1.25–2.73)	0.001
Platelets <$150\times10^3/\mu$L, n (%)	35 (42.7)	15 (22.1)	1.93 (1.14–3.28)	0.006
Elevated AFP >20 ng/mL, n (%)	58 (70.7)	31 (45.6)	1.55 (1.14–2.11)	0.002

* CI: Confidence Interval; AFP: Alpha-fetoprotein.

Table 6. Performance of non-invasive fibrosis markers for predicting advanced fibrosis (F3-F4)*.

Marker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC (95% CI)
APRI	>1.0	78.2	71.6	61.4	84.8	0.798 (0.725–0.871)
FIB-4	>2.67	81.8	68.4	59.2	86.7	0.812 (0.741–0.883)
FibroScan (kPa)	>9.5	94.5	91.6	86.7	96.7	0.958 (0.923–0.993)
Albumin (g/dL)	<3.5	72.7	77.9	64.5	83.6	0.785 (0.709–0.861)
Platelets ($\times 10^3/\mu$L)	<150	69.1	82.1	68.0	82.9	0.801 (0.728–0.874)

* PPV: Positive Predictive Value; NPV: Negative predictive value; AUROC: Area under the receiver operating characteristic curve; APRI: Aspartate aminotransferase to platelet ratio index.

DISCUSSION

This molecular epidemiological study outlined genotype-specific precore and BCP mutations in HBeAg-negative CHB patients in Iraq. Also, the association of these mutations with disease severity and clinical outcomes in Iraq was highlighted. It is known that genotype D is prevalent in the Middle Eastern

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Table 7. Composite risk score for predicting advanced hepatic fibrosis in patients with HBeAg-negative chronic hepatitis B*.

Variable	Points
G1896A mutation present	2
Core promoter mutations (A1762T/G1764A)	2
Albumin <3.5 g/dL	3
Platelets $<150\times10^3/\mu$L	3
Viral load >20,000 IU/mL	1
Age >50 years	1

* Risk categories: The chance of advanced liver fibrosis in patients with
- low risk (score 1-3) is 8.5%.
- Intermediate risk (score 4-7) is 42.3%.
- High risk (score 8-12) is 85.7%.

Composite Score AU-ROC 0.876 (95% CI 0.815-0.937)

region. Thus, there is a need for regional molecular profiling for better management.

The study supported that genotype D was most prevalent (85.3%). According to recent studies in Iraq, HBV has a similar pattern. For instance, Al-Suraifi et al. reported a 78% prevalence of genotype D in 2016 [9]. Moreover, neighboring countries display similar patterns. Notably, Iran (82%) [11] and Saudi Arabia, which has been associated with like frequencies of mutations and association with disease progression [12].

The higher viral loads seen in genotype D infections imply greater replicative fitness, perhaps due to regulatory differences. Research from Turkey and Iran recorded similar differences in viral load [13–15].

The reason why this variant can replicate at a higher efficiency may involve a better interaction of the polymerase variant, which is specific to genotype D, with the host cell machinery and more effective transcription due to adapted promoter sequences [16].

We found that 61.7% of all genotype D patients had the G1896A mutation. This is one of the highest prevalences reported in the Middle East. Further, it is more than what is seen in East Asian populations [17]. The form of nucleotide position (1858) is vital as it pairs with nucleotide position (1896) in the precore stem-loop structure. The presence of thymidine at (1858) helps in the formation of a stabilizing stem-loop structure with adenine at (1896) in genotype D that facilitates selection of and helps the G1896A mutation to persist [18].

The G1896A mutation has a strong relationship with advanced hepatic fibrosis with an adjusted OR of 3.1. Thus, G1896A has important clinical implications for risk stratification and monitoring strategies. This association probably represents several interlinked pathogenic mechanisms, including changes in viral protein expression patterns, augmented cytotoxic T cells responses toward hepatocytes producing mutant viral proteins, and possibly direct cytopathic effects through disrupted viral-host protein interactions [19]. An even stronger association was seen with the combination of BCP mutations (OR=4.3), possibly due to synergistic effects on disease progression. Above all, the combination of BCP mutations could enhance viral replication and alter immune responses [20].

The G1896A mutation was more common in advanced hepatic fibrosis stage. This means that the mutation plays a role in worsening the disease, rather than just being related to the disease. Longitudinal studies from other populations have confirmed this pattern, showing temporal relationships between the emergence of the mutations and the acceleration of liver fibrosis [21]. This association at a molecular level may arise from changes in pathways involved in apoptosis of hepatic cells, increased stellate cells activation, and impairment of liver regeneration [22].

The study reported that simple lab parameters were effective in predicting hepatic fibrosis. Having an albumin level below 3.5 g/dL or a platelet count below 150,000 is strong predictors of liver fibrosis. Therefore, these parameters are of value in limited resources setting. Access to advanced diagnostics tools remains limited in Iraq, this implementation addresses such problem [23]. By combining these parameters with mutation screening in high-risk patients, we can limit costs and maximize accuracy.

Worldwide, the association of the G1896A mutation with liver fibrosis severity has been reported. Moreover, application of the data and biotechnology with artificial intelligence in the health care system might improve the diagnostic accuracy and therapeutic responses, particularly in limited-resources regions [24].

The Iraqi people might be genetically different with some HLA alleles that could be related to the progression of HBV. Moreover, other environmental cofactors, including dietary habits and exposures to hepatotoxins can play a role too [25].

Patients with G1896A mutations and negative mutations show different responses to treatment. This reveals how important mutation characterization is. Reduced viral response rates in patients with specific mutations indicate that treatment strategies will need to be adapted. Treatment strategies may involve the use of higher dosing, combination treatments, or varying antiviral agents which target the specific viral house-keeping mutation [26]. New treatments that target specific mutations show great promise for patients with HBV infections with difficult-to-treat mutations [26].

The current study utilized a composite risk score, which, upon validation, represents a practical clinical tool integrating readily available laboratory parameters with selected molecular diagnostic markers [27]. The score demonstrated strong discriminatory ability (AUROC = 0.876), supporting its usefulness in identifying patients who require closer monitoring and early intervention [28, 29]. Its implementation could enhance risk stratification across the healthcare system and promote more efficient allocation of limited medical resources.

The study's results extend beyond individual patient management to inform public health policy. Given the high prevalence of genotype D and its associated mutations, implementing genotype-based screening programs and developing treatment guidelines tailored to Iraq's molecular profile would be a prudent step toward improving disease control and clinical outcomes. We could improve early identification of high-risk patients and optimize long-term outcomes through integrating mutation screening into national HBV management protocols [30].

Moreover, our findings showed that as fibrosis stages progress (44.2% in F0-F1 to 76.5% in F4), G1896A mutation enrichment ascends together with HBsAg levels, complementing its known association with a lower risk for hepatocellular carcinoma development, enabling risk stratification in clinical practice.

This study has several limitations. The study design does not allow for establishing temporal or causal links between viral mutants and disease progression. Recruiting from a single city may hamper the ability to generalize the findings to other Iraqis. The low statistical power for inter-genotype comparisons was partly due to the small size of the non-genotype D group (n=22). The associations reported may have been confounded by unmeasured host genetics, environment, and treatment histories. lastly, the composite risk score was validated internally; however, external validation in larger multicenter cohorts is essential for clinical use.

CONCLUSION

The present study shows that HBV genotype D is predominates among Iraqi HBeAg-negative CHB patients and is strongly associated with the high prevalence of G1896A precore mutation, which is a strong independent predictor of advanced hepatic fibrosis and complications. The impact of precore and BCP mutations on disease progression underscores the need for molecular profiling for HBV risk assessment. Using the information available through basic lab testing alongside mutation analysis can help identify high-risk patients easily. The results suggest that treatment strategies based on genotype and mutation could improve outcomes in this group. Future studies must assess the mutations over the long term and further to evaluate mutation-specific therapies and develop cost-effective screening algorithms for wider use in endemic populations.

ETHICAL DECLARATIONS

Acknowledgments

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Ethics Approval and Consent to Participate

The study was approved by the Ethical Approval Committee of the University of Anbar (Reference number 38, dated 2-5-2025), with all procedures adhering to the last updated version (October 2024) of the Declaration of Helsinki principles. Informed consent was obtained from all participants.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Artificial Intelligence

The authors used artificial intelligence for minor language editing; the authors did all scientific content and data interpretation.

Authors' Contributions

Jassim HS: Conceptualization, methodology, formal analysis, and writing-original draft. Khalil MA: Supervision, investigation, data curation, and writing-review and editing (final draft). Majeed YH: Supervision, project administration, funding acquisition, and writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

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