Original Article

Lower prevalence of hepatic fibrosis in low viremic hepatitis B patients with fluctuating HBV DNA levels

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Abstract

Background: In chronic hepatitis B virus (HBV) patients, fluctuations in HBV DNA serve as a "gray area" and impede the accurate identification of inactive carriers. We aimed to assess if such fluctuations impact the presence of significant hepatic fibrosis (Metavir F2-4) in chronic HBV patients.

Methods: Consecutive, untreated HBeAg-negative carriers ($n = 234$) with fluctuating HBV DNA ($n = 73$) above or below a level of 2000 IU/mL were included and compared to those without fluctuations ($n = 161$). Patients without fluctuating HBV DNA were further analyzed based on those with persistently low (<2,000 IU/mL, $n = 137$) and higher HBV DNA (2,000–20,000 IU/mL, $n = 24$). Hepatic fibrosis (assessed by transient elastography) was correlated with virologic and biochemical profiles.

Results: The mean age of the overall cohort was 47.8 ± 11.1 years, of whom 107 (45.7%) were male. During a median of 60 months (interquartile range [IQR] 34–82) of follow-up, 73 (31.2%) patients had a mean of 1.6 ± 0.9 fluctuations in HBV DNA. The median time to the first fluctuation was at 14.5 (IQR 5.0–33.7) months. Patients with fluctuating viremia had higher $\log_{10}$ qHBsAg (3.1 ± 0.8 vs. 2.7 ± 1.0, $P = 0.022$) and HBV DNA (3.4 ± 0.5 vs. 2.7 ± 0.8, $P < 0.001$) compared to those without fluctuations. Patients with fluctuant viremia were less likely to have F2-4 fibrosis (8.2%) compared to those without fluctuant viremia (18.2%, odds ratio [OR]: 0.407, 95% confidence interval [CI]: 0.161–1.030; $P = 0.052$). Males tended to have less fluctuation constituting 37.0% of patients with fluctuating HBV DNA ($P = 0.071$). Fluctuations occurred more frequently in those with predominantly higher HBV DNA levels (26.0%) compared to those without fluctuations (14.9%; $P = 0.030$).

Conclusions: Fluctuating HBV DNA levels occur frequently but are not associated with significant fibrosis. Minor fluctuations in HBV DNA levels are unlikely to be of clinical relevance.

Keywords: Fluctuation, gray zone, HBV DNA, hepatitis B, inactive carriers, significant fibrosis, viremia

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INTRODUCTION

Hepatitis B virus (HBV) chronically infects approximately 292 million people globally and is a leading cause of cirrhosis, hepatocellular carcinoma (HCC), and liver-related death.[1] Assessment of hepatic fibrosis is of immense importance in managing chronic HBV patients. During the HBeAg-negative phase, treatment is recommended when alanine aminotransferase (ALT) is persistently or intermittently elevated above the upper limit of normal (ULN), the HBV DNA is over 2,000 IU/mL, and/or with significant fibrosis (for instance, METAVIR score F2-4).[2,3] In contrast, given the good long-term prognosis, periodic monitoring is recommended in patients with chronic HBV infection ("inactive carriers").[2,3]

It is recognized that HBeAg-negative chronic hepatitis B (CHB) can run a fluctuating course[4,5] with variability in HBV DNA and ALT levels, over time. Although there is a reasonable body of literature on liver histological changes in HBV cohorts with persistently normal ALT,[6-8] studies focusing on fluctuations in HBV DNA levels are scarce, with none assessing the development of liver fibrosis. Significant fibrosis in patients with low viremia may occur, with reports of progression to cirrhosis.[6,7] Because fluctuations of ALT, serum HBV DNA or both may occur, the distinction of true inactive carriers from HBeAg-negative CHB may sometimes be difficult. These findings call into question the validity of conventional staging of HBV, in large part because of the substantial proportion of patients who do not fit readily into one of the usual stages or phases, and represent "gray zones" of disease classification.[2,9,10] We have previously reported fluctuations in HBV DNA levels above or below 2,000 IU/mL in 33.8% of HBeAg-negative patients[8] although the impact of this fluctuation was not studied, particularly in relation to the development of liver fibrosis.

Little is known about the natural history of patients with fluctuant viremia because appropriate studies including large cohorts are lacking. Consequently, whether these patients simply deserve routine long-lasting monitoring or more intense monitoring with active intervention remains unclear. We aimed to assess the prevalence of HBV DNA fluctuation in an unselected cohort of HBeAg-negative patients with low viremia, and the validity of categorizing CHB into phases based upon measures of hepatic fibrosis and viral load, assuming that such determination will be useful for determining the need for antiviral therapy.

PATIENTS AND METHODS

Study patients

This study included all untreated, consecutive HBeAg-negative patients from the outpatient clinics of King Abdulaziz Medical City in Jeddah and Riyadh between May 2016 and January 2021. Patients were identified from a search of clinical records and the Saudi Observatory Liver Disease (SOLID) registry, which is a prospective, multicenter observational registry. The two recruiting centers provide healthcare to Saudi citizens exclusively, and to Saudi Arabian National Guard members and their families predominantly. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the Principles of Good Clinical Practice and the Institutional Review Boards of both centers approved the study.

Eligible patients for the analysis had detectable HBsAg for at least 6 months before inclusion in the study. All patients were HBeAg-negative, had HBV DNA values predominantly <20,000 IU/mL ($n = 234$), and were between 16 and 80 years of age. The median duration of follow-up for the entire cohort was 60 months (interquartile range [IQR] 34–82). The exclusion criteria were (i) co-infection with hepatitis C, delta virus, or HIV; (ii) superimposed with other liver diseases; (iii) antiviral, immunosuppressive, or hepatotoxic medications; (iv) decompensated cirrhosis with a Child-Pugh score >6, or evidence of portal hypertension, variceal bleeding, laboratory findings of a platelet count <150 (10^9/L), an international normalized ratio ≥1.3; (v) creatinine >135 μmol/L; (vi) presence of hepato-biliary malignancy; (vii) alcohol consumption >20 g/day; and (viii) organ transplantation.

Study design and definitions

Patients were classified as either predominantly low HBV DNA where the majority of values were <2,000 IU/mL, or high when the majority of values were between 2,000 and 20,000 IU/mL. The fluctuation was defined as any ≥1 crossover of HBV DNA from a level above or below a threshold of 2,000 IU/mL. HBV DNA levels were defined as persistently low when all levels were <2,000 IU/mL and defined as persistently high when values were in the range of 2,000–20,000 IU/mL, over ≥12 months. A minimum of three HBV DNA measurements was required and the reference value utilized for analysis was the last level before the performance of transient elastography (TE) for liver stiffness measurement (LSM). A median number of 6 [IQR 4–9] measurements were made for 60 months.

A minimum number of three ALT measurements were required, and the last level before the performance of

TE was used as the index value. A median number of 10 [IQR 7–15] ALT recordings were made for 60 months for the overall cohort. Routine liver biochemical tests were performed using commercially available autoanalyzers and hepatitis serological markers were assayed using commercially available enzyme-linked immunoassays. Quantitative HBV DNA levels were measured by Abbott Real-Time HBV assay (Abbott Molecular, Inc., Des Plaines, IL, USA), with a lower detection limit of 10 IU/mL. The serum quantitative HBsAg levels (qHBsAg) were measured by the Abbott ARCHITECT® Assay (Architect i2000SR, Abbott Diagnostics; Abbott Laboratories, Chicago, IL, USA). The detection value of qHBsAg ranged from 0.05 to 250 IU/mL and samples with HBsAg titers >250 IU/mL required a 1:500 dilution.

Liver fibrosis assessment

All patients underwent TE for LSM (FibroScan® 502 Touch, Echosens, Paris, France), and the procedure was performed by a single operator at each site with experience of over 500 procedures, according to instructions provided by the manufacturer. All TE assessments were made from January 2018 to December 2020. The median value of multiple successful measurements was recorded as the LSM value for each patient. Only LSM processes with at least 10 validated measurements, an IQR less than 30% of the median value, and a success rate of at least 60% were considered reliable.[11,12] Stiffness is expressed in kilopascals (kPa). Any LSM value that did not satisfy the aforementioned conditions was considered unreliable and therefore excluded from further analysis. An XL probe was used as a second-line probe for patients for whom the regular M probe failed, such as some obese patients or patients with BMI ≥30 kg/m^2.[13] The final controlled attenuation parameter (CAP) was taken as the median of individual CAP and is expressed in dB/m. Previously described cut-offs to discard moderate fibrosis (F ≥2) was TE ≤6 kPa. Steatosis scores of ≥2 were defined as moderate–severe corresponding to CAP values >260 dB/m.

Statistical analysis

The sampling technique was convenient sampling, and it was chosen to ensure the availability of required data. Patients with fluctuating HBV DNA above or below a level of 2,000 IU/mL were compared to those without fluctuations. Quantitative variables are expressed as the mean ± standard deviation or the median with IQR (25th–75th percentile), and categorical variables as frequencies and proportions. The unpaired Student's *t*-test was used to compare the means or log means of the variable, if the variable was normally or not normally distributed, respectively. Mann–Whitney *U* test was used to compare the medians of two groups when the variables were not normally distributed. The Chi-square or Fishers' exact test ($\alpha = 0.05$) was used to compare frequencies and proportions in categorical variables, as appropriate. Odds ratios (OR) were calculated where appropriate, along with their 95% confidence intervals (CI). All analyses related to HBV DNA were conducted after logarithmically transforming the values to account for skewed distribution. Statistical Package for Social Sciences (*SPSS, version* 21.0; Chicago, IL, USA) was used for data analysis.

RESULTS

Baseline characteristics of patients

The mean age of the overall cohort was 47.8 ± 11.1 years, of whom 107 (45.7%) were males and 44 (18.8%) had elevated ALT. The mean HBV DNA $\log_{10}$ was 2.9 ± 0.8, and 137 (58.5%) had persistently low HBV DNA (<2,000 IU/mL) and 24 (10.3%) had persistently high HBV DNA (>2,000 IU/mL). During a median of 60 months (IQR 34–82) of follow-up, 73 (31.2%) patients had a mean of 1.6 ± 0.9 fluctuations in HBV DNA. Patients with fluctuations had more HBV DNA measurements (8 [IQR 5–9.5]) compared to those without fluctuations (6 [IQR 4–9]; $P = 0.001$). Throughout the follow-up, most patients exhibiting fluctuant viremia had a single fluctuation ($n = 43$, 18.8%), whereas two were seen in 16 (6.8%), three in 11 (4.7%), and four fluctuations in 3 patients (1.3%). Most fluctuations occurred early during the follow-up, with a median occurrence of 14.5 (IQR 5.0–33.7) months [Figure 1]. The mean magnitude of fluctuation was $\log_{10}$ 3.5 ± 0.5 Fluctuations >20,000 IU/mL occurred in 11 (4.7%) patients, occurring usually in those with persistently higher (2,000–20,000 IU/mL) HBV DNA levels (9/11, 81.8%) and were mostly transient. Co-morbid conditions were observed in a majority of the

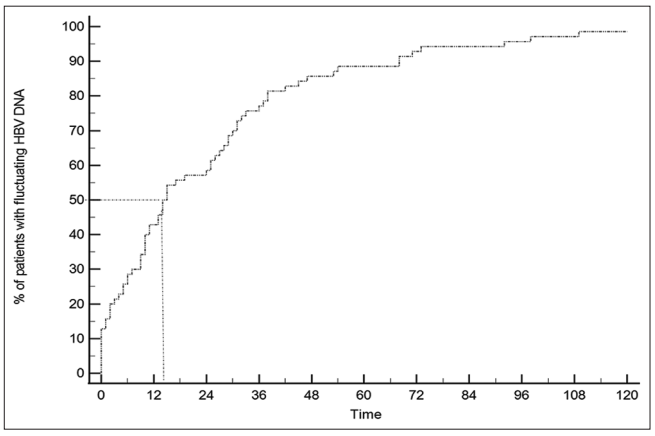

Figure 1: Kaplan–Meir curve displaying overall incidence of HBV DNA fluctuation. Median fluctuation occurred at 14.5 months (IQR: 5.0–33.7)

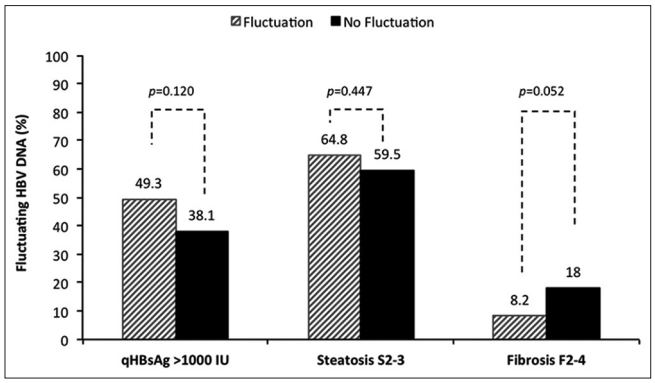

Figure 2: Relationship of fluctuating HBV DNA levels with high quantitative HBsAg levels, moderate–severe steatosis, and significant fibrosis

patients, including diabetes in 19.7% and dyslipidemia in 24.9%. Moderate–severe steatosis (S2-3) was observed in 140 patients (61.1%) and 35 (15.0%) had significant fibrosis stage F2-4 (data not shown).

Effect of fluctuating viremia

Overall, patients with fluctuation had a longer duration of follow-up (66.0 [IQR 43–84] months) compared to those without fluctuations (56.5 [IQR 28.3–80.0] months; $P = 0.023$). The presence of F2-4 fibrosis in those with fluctuation (8.2%) was lower but not significantly different (OR: 0.407, 95% CI: 0.161–1.030; $P = 0.052$) from those without fluctuation [18.0%, Figure 2]. Similarly, F2-4 fibrosis was not different (OR: 0.640, 95% CI: 0.275–1.487; $P = 0.345$) in those with predominantly lower (16.1%) or higher HBV DNA [9.5%, Table 1]. The presence of F2-4 fibrosis was not different in relation to the number of fluctuations [$P = 0.346$; Table 2]. Males tended to have less fluctuation constituting 37.0% of patients with fluctuating HBV DNA ($P = 0.071$). Patients with fluctuations tended to have higher qHBsAg $\log_{10}$ (3.1 ± 0.8 vs. 2.7 ± 1.0; $P = 0.022$) and were more likely to have predominantly higher HBV DNA levels (26.0% vs. 14.9%; $P = 0.030$) compared to those without fluctuations [Table 1]. These differences in qHBsAg $\log_{10}$ ($P < 0.002$) and HBV DNA $\log_{10}$ ($P < 0.001$) were retained when patients were categorized on the basis of fluctuating vs. persistently low or high HBV DNA [Supplementary Table 1].

Effect and outcome of quantitative HBsAg levels

Higher qHBsAg levels were identified by younger age ($P < 0.001$), presence of diabetes ($P = 0.002$), and higher HBV DNA $\log_{10}$ ($P < 0.001$), >2,000 IU/mL ($P = 0.001$), and steatosis grade 2–3 ($P = 0.042$). Higher qHBsAg levels did not impact the presence of fluctuations in HBV DNA [$P = 0.120$, Figure 3]. Fibrosis 2–4 was not significantly different (OR: 0.807, 95% CI: 0.384–1.701; $P = 0.574$) in patients with high qHBsAg (14.1%) compared to lower levels [16.9%, Table 3].

Factors identifying significant fibrosis

Patients with significant (F2-4) fibrosis were identified by lower serum albumin levels, higher BMI, and ALP, AST, and HBV DNA levels [$P < 0.05$ for all, Supplementary Table 2]. These patients had similar age, diabetes, dyslipidemia, hepatic steatosis, or HBV DNA values >2,000 IU/mL compared to those with non-significant

Table 1: Baseline characteristics of chronic HBV patients in relation to fluctuating or non-fluctuating HBV DNA

Variable	Overall (*n*=234)	Fluctuating HBV DNA (*n*=73)	Nn fluctuating HBV DNA (*n*=161)	*P*
Age (years)	47.8±11.1	46.6±12.1	48.3±10.6	0.276
Male gender	107 (45.7)	27 (37.0)	80 (49.7)	0.071
BMI (kg/m^2)	30.0±6.4	29.9±6.6	30.0±6.3	0.955
Diabetes mellitus	46 (19.7)	14 (19.2)	32 (20.0)	0.884
Dyslipidemia	58 (24.9)	16 (21.9)	42 (26.3)	0.478
AFP	5.2±11.4	5.0±6.5	5.3±13.1	0.835
Platelets	257.2±76.1	262.6±71.8	254.7±78.1	0.469
PT	12.2±2.2	11.9±0.9	12.4±2.4	0.113
ALP	81.2±32.0	78.7±20.5	82.4±36.1	0.418
Albumin	41.6±3.1	41.8±2.5	41.5±3.3	0.615
qHBsAg $\log_{10}$	2.8±1.0	3.1±0.8	2.7±1.0	0.022
AST	23.0±14.8	21.3±7.8	23.8±17.0	0.228
Elevated AST >37	17 (7.3)	5 (6.8)	12 (7.5)	0.869
ALT	26.9±21.0	26.9±25.7	26.9±18.6	0.995
Elevated ALT >40	44 (18.8)	12 (16.4)	32 (19.9)	0.533
HBV DNA $\log_{10}$	2.9±0.8	3.4±0.5	2.7±0.8	<0.001
Predominant HBV DNA				
Low (<2000 IU/mL)	191 (81.6)	54 (74.0)	137 (85.1)	
High (>2000 IU/mL)	42 (17.9)	19 (26.0)	24 (14.9)	0.030
Steatosis (CAP)	266.0±56.3	272.1±53.6	263.3±57.4	0.280

Data are expressed as mean±standard deviation or *n* (%) as appropriate. n; number. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. PT: prothrombin time, AFP:α-feto-protein. BMI: body mass index. qHBsAg: quantitative hepatitis B surface antigen, HBV: hepatitis B virus

Table 2: Distribution of significant fibrosis based on the presence and number of fluctuations

Number of fluctuations	Fibrosis stage	
	F0-1	F2-4
0	132 (65.8)	29 (82.9)
1	41 (21.1)	2 (5.7)
2	13 (6.5)	3 (8.6)
3	10 (5.0)	1 (2.9)
4	3 (1.5)	0 (0.0)

Data are expressed as *n* (%), Distribution of fibrosis was similar across fluctuating numbers (P=0.346)

fibrosis. The mean fluctuations were not different in those with significant fibrosis (1.83 ± 0.75) compared to those without (1.63 ± 0.90; P = 0.589).

DISCUSSION

In this real-life cohort study, we investigated for the first time the performance of repeated HBV DNA measurements for the identification of fluctuant viremia and its association with the presence of hepatic fibrosis. Although viral fluctuation at a threshold of 2,000 IU/mL was a frequent occurrence, occurring in one-third of the individuals, it was not associated with the presence of significant fibrosis. Despite fluctuations occurring frequently in those with low HBV DNA levels (<2,000 IU/mL), a substantially lesser number of such individuals harbored significant fibrosis (8.2%) compared to those with non-fluctuant, persistently low HBV DNA levels (18.1%).

Table 3: Comparison of HBV patients with low or high quantitative HBsAg

Variable	qHBsAg <1000 (*n*=130)	qHBsAg >1000 (*n*=92)	*P*
Age (years)	51.0±11.1	43.7±9.8	< 0.001
Male gender	56 (43.1)	46 (50.0)	0.308
BMI (kg/m^2)	30.5±6.2	29.1±6.8	0.131
Diabetes mellitus	34 (26.4)	9 (9.8)	0.002
Dyslipidemia	37 (28.7)	18 (19.6)	0.122
AFP	5.2±7.2	3.9±5.0	0.146
Platelets	257.2±85.1	259.0±65.9	0.860
PT	12.1±1.4	12.1±1.9	0.993
ALP	83.2±38.0	79.0±22.7	0.353
Albumin	41.5±3.1	41.7±3.2	0.557
AST	22.2±15.3	24.6±14.8	0.245
Elevated AST>37	9 (6.9)	8 (8.7)	0.625
ALT	26.8±24.9	27.3±15.6	0.863
Elevated ALT>40	23 (17.7)	20 (21.7)	0.452
HBV DNA Log_{10}	2.7±0.7	3.1±0.8	< 0.001
Predominant HBV DNA			
Low (<2000 IU/mL)	117 (90.0)	67 (72.8)	
High (>2000 IU/mL)	13 (10.0)	25 (27.2)	0.001
Fluctuating HBV DNA	34 (26.2)	33 (35.9)	0.120
Steatosis (CAP)	272.7±57.2	256.2±52.2	0.032
Steatosis 2-3	85 (67.5)	49 (53.8)	0.042
Fibrosis 2-4	22 (16.9)	13 (14.1)	0.574

Twelve patients in our cohort did not have qHBsAg levels available and were excluded in this analysis. Data are expressed as mean ± standard deviation or n, number (%) as appropriate. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. PT: prothrombin time. AFP:-feto-protein. BMI: body mass index. qHBsAg: quantitative hepatitis B surface antigen. HBV: hepatitis B virus

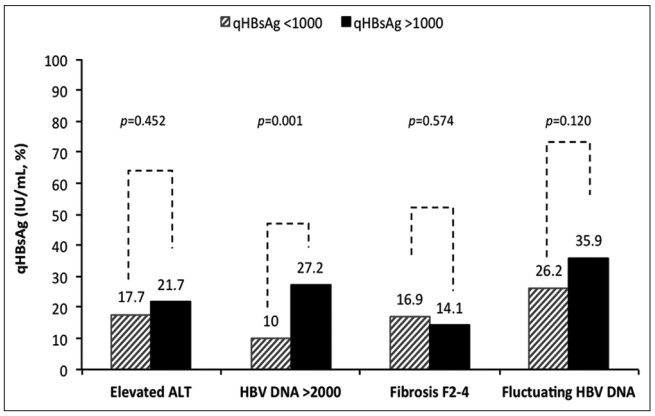

Figure 3: Relationship of high quantitative HBsAg levels with serum ALT, fluctuating HBV DNA, HBV DNA levels, and significant fibrosis

Proper categorization of HBV-infected patients aids in the allocation of antiviral therapy and thus helps in reducing the associated long-term complications. Although the traditional classification of CHB is an important clinical distinction, some individuals fall into an indeterminate area, or "gray zone," requiring treatment decisions to be individualized.[2] Patients with fluctuant viremia constitute one such category of "gray zone" HBV carriers where the clinical distinction between inactive carriers and HBeAg-negative CHB becomes blurred. In our study of this particular category of "gray zone" patients, we have shown that individuals with fluctuant viremia hold no special distinction and have clinical, biochemical, and histological characteristics similar to "inactive carriers." Our findings are similar to other studies dealing with "gray zone" individuals. Bonacci *et al.*[9] described different categories of "gray zone" carriers including individuals falling between inactive carrier state and HBeAg-negative CHB, and reported excellent long-term outcomes in the absence of treatment, with a low rate of progression to HBeAg-negative CHB. Similarly, Oliveri *et al.*[14] also reported a benign outcome in a group of low viremic active carriers (HBV DNA ≤20,000 IU/mL), where only one patient (2.2%) progressed to the chronic hepatitis phase, and no major liver-related events were reported during the study.

The vast majority of our patients tended to exhibit early fluctuations, with roughly half occurring within the first 2 years of follow-up. Most (60%) demonstrated a single fluctuation over the median 5 years of follow-up, and with no disease impact demonstrated by multiple fluctuations.

The management of HBeAg-negative CHB patients who have HBV DNA levels <20,000 IU/mL remains a matter

of debate. A small proportion with HBV DNA <2,000 and those with HBV DNA between 2,000 and 20,000 IU/mL exhibit significant fibrosis and hence remain at a greater risk for disease progression.[6-8] In our study, the presence of hepatic fibrosis was similar in individuals with HBV DNA persistently <2,000 and 2,000–20,000 IU/mL, whereas it tended to be less frequent in those who demonstrated fluctuant levels. Despite a higher qHBsAg and HBV DNA $\log_{10}$ in patients with fluctuant viremia, significant fibrosis tended to be less frequent. Although this distinction remains a point of clinical interest, it is unclear what mechanisms are responsible for this observation. There were no obvious predictors of virologic fluctuation. Moreover, the only potential etiological basis for significant fibrosis was the presence of a greater BMI, hinting at the role of coexistent non-alcoholic fatty liver disease as a factor for the increased rates of hepatic fibrosis development. However, in patients with fluctuant and non-fluctuant viremia no differences in BMI, diabetes, or hepatic steatosis were seen. More relevantly, in multiple cohorts of HBV assessed histologically, the presence of hepatic steatosis revealed conflicting results on the occurrence of significant fibrosis.[8,15,16] Nevertheless, a greater prevalence of co-morbidities were observed in individuals with CHB correlated with liver disease-related sequelae in patients from Saudi Arabia.[17] As such, a longer duration of follow-up is warranted to further understand the role of underlying co-morbidities in hepatic disease progression.

Previous studies have evaluated the value of single-point or repeated qHBsAg measurements to determine the identification of the inactive carrier phase through long-term follow-up. However, reports from different regions have been conflicting in their outcomes. Previous analyses have suggested a qHBsAg of 1,000 IU/mL as an appropriate cutoff to distinguish active from inactive HBV.[18,19] More recently, a multi-center analysis showed that the probability a patient would switch to active HBV at any following year was 24% for patients with initial qHBsAg levels >1,000 IU/mL.[20] However, in a cohort from Saudi Arabia, qHBsAg levels failed to accurately distinguish inactive carriers and were unable to identify significant histological disease.[21] Similarly, in this analysis, a threshold qHBsAg levels >1,000 IU/mL failed to distinguish individuals with significant fibrosis or identify those with HBV DNA fluctuations in a meaningful manner. Additionally, a substantial 73% of individuals with HBV DNA levels >2,000 IU/mL did not have qHBsAg levels >1,000 IU/mL, showing a poor correlation between these proposed thresholds. Nonetheless, it remains to be seen whether a long-term longitudinal follow-up at varying thresholds would be better for disease classification in Middle Eastern patients.

Our analysis must be viewed in the context of its limitations. First, this was a retrospective real-life cohort, limited by a lack of pre-defined serial performances of TE to assess the dynamic nature of liver fibrosis evolution. Second, it is our practice to monitor patients on a 6-month basis after an initial classification based on liver enzymes and HBV DNA levels.[3,22] However, HBV DNA levels may fluctuate more frequently, especially those above or below a threshold of 2,000 IU/mL. As such, it is feasible that some HBV DNA fluctuations may have been missed, and some individuals potentially misclassified. Third, TE does not represent the gold standard for liver fibrosis assessment. However, we mitigated the role of inter-operator variability in LSM by restricting the performance of TE to single operators in either center throughout the study, each with vast experience in performing LSMs. In addition, it is noteworthy that in a recent prospective study, excellent diagnostic accuracy was reported using TE compared to biopsy with an area under the receiver operating characteristic curve of 0.81 for ≥F2, 0.93 for ≥F3, and 0.93 for F4.[23] Based on these findings, recent guidelines have suggested diagnostic algorithms that incorporate liver stiffness measurement as an important part rather than liver biopsy to facilitate the categorization of patients in a more rapid and less invasive way.[2,3] This is especially relevant for patients who are thought to be "inactive carriers" in whom the risk of a liver biopsy may not be justified.

In conclusion, fluctuations in HBV DNA levels occur frequently but do not distinguish patients with a higher prevalence of significant fibrosis. Minor fluctuations are unlikely to be of clinical relevance, wherein such patients may be less likely to have significant fibrosis compared to those with non-fluctuant viremia. Such "gray zone" patients could potentially be classified as true inactive HBV carriers.

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Conflicts of interest

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REFERENCES

1. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: A modelling study. Lancet Gastroenterol Hepatol 2018;3:383-403.
2. European Association for the Study of the Liver.EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection.J

Hepatol 2017;67:370-98.
3. Abaalkhail FA, Al-HamoudiWK, Khathlan A, Alghamdi S, Alghamdi M, Alqahtani SA, *et al.* SASLT practice guidelines for the management of Hepatitis B virus-An update. Saudi J Gastroenterol 2021;27:115-26.
4. Kwon H, Lok AS. Hepatitis B therapy. Nat Rev Gastroenterol Hepatol 2011;8:275–84.
5. Papatheodoridis GV, Chrysanthos N, Hadziyannis E, Cholongitas E, Manesis EK. Longitudinal changes in serum HBV DNA levels and predictors of progression during the natural course of HBeAg-negative chronic hepatitis B virus infection. J Viral Hepatol 2008;15:434–41.
6. Sanai FM, Helmy A, Bzeizi KI, Babatin MA, Al-Qahtani A, Al-Ashgar HA, *et al.* Discriminant value of serum HBV DNA levels as predictors of liver fibrosis in chronic hepatitis B. J Viral Hepat 2011;18:e217–25.
7. Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, *et al.* Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology 2008;134:1376–84.
8. Abdo AA, Bzeizi KI, Babatin MA, AlSohaibani F, AlMana H, Alsaad KO, *et al.* Predictors of significant fibrosis in chronic hepatitis B patients with low viremia. J Clin Gastroenterol 2014;48:e50-6.
9. Bonacci M, Lens S, Mariño Z, Londoño MC, Rodríguez-Tajes S, Mas A, *et al.* Anti-viral therapy can be delayed or avoided in a significant proportion of HBeAg-negative Caucasian patients in the Grey Zone. Aliment Pharmacol Ther 2018;47:1397-408.
10. Vlachogiannakos J, Papatheodoridis GV. Hepatitis B: Who and when to treat? Liver Int 2018;38:71-8.
11. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835-47.
12. Kao WY, Chang IW, Chen CL, Su CW, Fang SU, Tang JH, *et al.* Fibroscan-based score to predict significant liver fibrosis in morbidly obese patients with nonalcoholic fatty liver disease. Obes Surg 2020;30:1249-57.
13. Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, *et al.* Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012;55:199-208.
14. Oliveri F, Surace L, Cavallone D, Colombatto P, Ricco G, Salvati N, *et al.* Long-term outcome of inactive and active, low viraemic HBeAg-negative-hepatitis B virus infection: Benign course towards HBsAg clearance. Liver Int 2017;37:1622-31.
15. Baclig MO, Reyes KG, Liles VR, Mapua CA, Dimamay MP, Gopez-Cervantes J. Hepatic steatosis in chronic hepatitis B: Astudy of metabolic and genetic factors. Int J Mol Epidemiol Genet 2018;9:13-9.
16. Mak LY, Hui RW, Fung J, Liu F, Wong DK, Cheung KS, *et al.* Diverse effects of hepatic steatosis on fibrosis progression and functional cure in virologically quiescent chronic hepatitis B. J Hepatol 2020;73:800-6.
17. Sanai FM, Alghamdi H, Alswat KA, Babatin MA, Ismail MH, Alhamoudi WK, *et al.* Greater prevalence of comorbidities with increasing age: Cross-sectional analysis of chronic hepatitis B patients in Saudi Arabia. Saudi J Gastroenterol 2019;25:194-200.
18. Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, *et al.* Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 2010;139:483-90.
19. Martinot-Peignoux M, Lapalus M, Laouénan C, Lada O, Netto-Cardoso AC, Boyer N, *et al.* Prediction of disease reactivation in asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline serum measurements of HBsAg and HBV-DNA. J Clin Virol 2013;58:401-7.
20. Brouwer WP, Chan HL, Brunetto MR, Martinot-Peignoux M, Arends P, Cornberg M, *et al.* Repeated measurements of Hepatitis B surface antigen identify carriers of inactive HBV during long-term follow-up. Clin Gastroenterol Hepatol 2016;14:1481-9.e5.
21. Ahmed FA, Bajaifar MS, Ahmed MA, Alalwan A, Sanai FA, Albeladi K, *et al.* Quantitative HBsAg levels do not identify hepatic fibrosis in HBeAg-negative chronic hepatitis B patients. Saudi J Gastroenterol 2019;25:286-92.
22. Aljumah AA, Babatin M, Hashim A, Abaalkhail F, Bassil N, Safwat M, *et al.* Hepatitis B care pathway in Saudi Arabia: Current situation, gaps and actions. Saudi J Gastroenterol 2019;25:73-80.
23. Marcellin P, Ziol M, Bedossa P, Douvin C, Poupon R, de Lédinghen V, *et al.* Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. Liver Int 2009;29:242-7.

Supplementary Table 1: Comparison of patients with persistently high, low or fluctuating HBV DNA

Variable	Fluctuating HBV DNA (*n*=73)	Non Fluctuating HBV DNA		*P*
		<2000 (*n*=137)	>2000 (*n*=24)	
Age (years)	46.6±12.1	48.4±10.9	47.8±9.4	0.538
Male gender	27 (37.0)	69 (50.0)	11 (47.8)	0.192
BMI (Kg/m^2)	29.9±6.6	30.1±6.5	29.0±5.0	0.714
Diabetes mellitus	14 (19.2)	27 (19.7)	5 (21.7)	0.964
Dyslipidemia	16 (21.9)	35 (25.5)	7 (30.4)	0.686
AFP	5.0±6.5	4.5±6.6	3.9±2.5	0.769
Platelets	262.6±71.8	257.3±80.5	239.3±60.4	0.442
PT	11.9±0.9	12.2±1.9	13.3±4.9	0.026
ALP	78.7±20.5	84.3±38.1	71.2±17.8	0.140
Albumin	41.8±2.5	41.5±3.4	42.1±2.7	0.591
qHBsAg Log_{10}	3.1±0.8	2.7±1.0	3.2±0.9	0.002
qHBsAg >1000	33 (49.3)	43 (32.3)	16 (72.7)	0.001
AST	21.3±7.8	23.4±16.5	26.0±20.1	0.665
Elevated AST >37	5 (6.8)	9 (6.5)	3 (13.0)	0.553
ALT	26.9±25.7	26.7±18.8	27.8±17.6	0.899
Elevated ALT >40	12 (16.4)	26 (18.8)	6 (26.1)	0.537
HBV DNA Log_{10}	3.4±0.5	2.5±0.6	3.8±0.6	<0.001
Steatosis (CAP)	272.1±53.6	264.2±57.5	257.6±57.9	0.267
Steatosis 2-3	46 (64.8)	80 (59.3)	14 (60.9)	0.742
Fibrosis 2-4	6 (8.2)	25 (18.1)	4 (17.4)	0.139

Data expressed as mean±standard deviation or *n* (%) as appropriate. *n*; number. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. PT: prothrombin time, AFP: α-feto-protein. BMI: body mass index. qHBsAg: quantitative hepatitis B surface antigen, HBV: hepatitis B virus

Supplementary Table 2: Characteristics of patients with significant fibrosis (F2-4)

Variable	Fibrosis 0-1 (*n*=199)	Fibrosis 2-4 (*n*=35)	*P*
Age (years)	47.4±11.3	50.3±10.0	0.156
Male gender	92 (46.5)	15 (42.9)	0.712
BMI (Kg/m^2)	29.5±6.2	32.7±6.6	0.006
Diabetes mellitus	37 (18.7)	9 (25.7)	0.336
Dyslipidemia	50 (25.3)	8 (22.9)	0.763
AFP	4.6±6.7	8.6±2.5	0.060
Platelets	260.1±77.2	240.9±68.5	0.171
PT	12.2±2.2	12.1±2.2	0.750
ALP	79.5±31.2	91.6±35.3	0.041
Albumin	41.9±3.0	39.8±3.0	<0.001
qHBsAg Log_{10}	2.9±1.0	2.7±1.1	0.468
AST	22.0±14.6	28.7±14.4	0.013
Elevated AST >37	10 (5.0)	7 (20.0)	0.002
ALT	26.0±20.9	32.0±21.1	0.119
Elevated ALT >40	34 (17.1)	10 (28.6)	0.109
HBV DNA Log_{10}	2.9±0.7	2.6±0.8	0.005
Predominant HBV DNA			
Low (<2000 IU/mL)	161 (80.9)	31 (88.6)	0.345
High (>2000 IU/mL)	38 (19.1)	4 (11.4)	
Fluctuating DNA	67 (33.7)	6 (17.1)	0.052
Steatosis (CAP)	263.5±55.5	278.9±59.6	0.141
Steatosis 2-3	117 (60.0)	23 (67.6)	0.399

Data expressed as mean±standard deviation or *n* (%) as appropriate. *n*; number. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. PT: prothrombin time, AFP: α-feto-protein. BMI: body mass index. qHBsAg: quantitative hepatitis B surface antigen, HBV: hepatitis B virus