Impact of Liver Biopsy on the Decision to Treat Patients with Chronic Hepatitis B Genotype D Virus Infection

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Key Words

Genotype D · Histopathology · Fibrosis · Viral load · Transaminases · Therapy

Abstract

Objectives: Patients with chronic hepatitis B virus (HBV) may exhibit significant liver pathology despite alanine aminotransferase (ALT) and HBV DNA levels below the cutoff values advised by treatment guidelines. We evaluated candidacy for HBV therapy when baseline histopathological changes are taken into consideration. **Methods:** Clinical, biochemical, serological, virological, and histopathological (METAVIR score) data of 117 patients with HBeAg-negative chronic HBV genotype D were collected and analyzed. **Results:** Significant pathology ($\geq F2$ and/or $\geq A2$) and fibrosis ($\geq F2 \pm \geq A2$) were found in 73 (62.4%) and 59 (50.4%) patients, respectively. Based on HBV DNA ($>2,000$ IU/ml) and ALT levels $>2 \times 40$ U/l (the standard cutoff value), only 31 (26.5%) patients were candidates for therapy. This increased to 58 (49.6%) patients when the new ALT cutoff values (30 U/l for males, and 19 U/l for females) were applied. Relying on either $\geq F2$ and/or $A \geq 2$ or $\geq F2 \pm \geq A2$ increases the treatment candidacy to 73 (62.4%) and 59 (50.4%) patients, respectively. Also, when compared with standard ALT cutoff

values, applying both new ALT cutoff values with either significant pathology or fibrosis increases treatment candidacy to 28 (23.9%) and 42 (35.9%) patients, respectively. **Conclusion:** Liver pathology is more reliable than ALT and HBV DNA in the decision to treat patients with HBeAg-negative chronic HBV genotype D.

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Background

Hepatitis B virus (HBV) infection is a major cause of morbidity and mortality with an estimated 350–400 million chronically infected persons worldwide [1]. Chronic HBV infection in Mediterranean and Middle East countries including Egypt and Saudi Arabia, is characterized epidemiologically by (1) an intermediate endemicity (overall HBsAg prevalence of 6.7%) [1, 2], (2) prevalence of a HBeAg-negative variant in $>80\%$ of patients [2, 3] which represents the late phase of infection where HBV pre-/basic core promoter variants are predominant with reduced or abolished HBeAg expression [4, 5], and (3) predominant HBV genotype D [3] which is more often associated with HBeAg-negative variants [6] and more severe liver disease [7], which is characterized by persis-

Fig. 1. Flow chart of the patients included in the study. DM = Diabetes mellitus; HDV = hepatitis D virus; HCC = hepatocellular carcinoma.

tent viral replication, progression of liver disease, and early development of cirrhosis [8, 9].

The available HBV treatment guidelines are similar [10–13], despite geographical differences in the epidemiology of the disease [14]. According to the American Association for Study of Liver Diseases (AASLD) 2007 guidelines, the parameter used to determine candidacy for treatment of HBeAg-negative patients is HBV DNA $\geq 2,000$ IU/ml and alanine aminotransferase (ALT) $\geq 2 \times$ the upper limit of normal (ULN). However, patients with lower ALT and/or HBV DNA levels may have abnormal histology and are consequently at increased risk of chronic liver disease complications [13]. Therefore, the new guidelines recommended a new lower ULN ALT and aspartate aminotransferase (AST) of 30 U/l for men and 19 U/l for women [11, 13].

ALT and HBV DNA levels do fluctuate, necessitating frequent monitoring or performing liver biopsy to assess disease activity, grade of fibrosis, and eligibility for treatment [13]. Additionally, the REVEAL study has indicated that patients with HBV DNA $< 2,000$ IU/ml are also at risk of disease progression [15]. Moreover, assessing liver histopathology was advised by the AASLD for evaluation of histologic disease in chronic HBV patients presenting with normal ALT and low HBV DNA levels [11].

Therefore, the main objective of this study in patients with chronic HBV infection was to evaluate whether incorporation of liver histopathological changes affects the decision to treat chronic HBV, HBeAg-negative, genotype D, which is prevalent in the Middle East and Mediterranean area, and influences the candidacy to treat such patients compared to the current strategies that rely mainly on serum ALT and HBV DNA levels.

Patients and Methods

Patients

A flow chart of patients included in the study is shown in figure 1. Briefly, 592 chronic hepatitis B patients attending our Viral Hepatitis Clinic from January 2008 to May 2012 were found to have chronic HBV infection.

Exclusion Criteria

After excluding cases with concomitant HCV, HIV, hepatitis delta infections, and those with hepatocellular carcinoma, alcoholic liver disease, diabetes mellitus, and hyperlipidemia, we were left with 256 patients with isolated HBV infection. Only 161 patients remained after further exclusion of cases without liver biopsy (not done because of failure to obtain consent, clinical contraindication), those with nontypable HBV DNA, patients with previous anti-HBV therapy, and those with decompensated liver disease. After excluding patients positive for HBeAg and those with non-D genotypes, a total of 117 patients were included in this analysis.

Inclusion Criteria

The 117 patients enrolled in this study fulfilled the following inclusion criteria after obtaining institutional approval and written informed consent: HBsAg-positive test for > 6 months, HBeAg-negative or negative detectable HBV DNA by polymerase chain reaction, recently performed liver biopsy, determined HBV genotype, no prior treatment with interferon or nucleos(t)ide analogues, and absence of the above exclusion criteria.

Laboratory Tests

Routine liver biochemical tests were performed using commercially available autoanalyzers, and hepatitis serological markers were assayed using commercially available enzyme-linked immunoassays (Clinotech Diagnostics). According to the instructions of the manufacturer, the ULN for ALT was determined to be 40 U/l (Bicon Diagnostik, Germany). Quantitative HBV DNA levels were measured by a COBAS TaqMan System (Roche Diagnostics Corporation, Indianapolis, Ind., USA), which has a lower detection limit of 30 IU/ml. Genotyping was performed using an INNO-LiPA HBV genotyping assay (Innogenetics N.V., Ghent, Belgium).

Table 1. Subject characteristics (n = 117)

Variable	Result
Age, years	
Mean ± SD	36.6±11.6
Median (range)	37 (14–70)
Age group, n (%)	
≤40 years	80 (68.4)
>40 years	37 (31.6)
Sex, n (%)	
Male	97 (82.9)
Female	20 (17.1)
ALT, U/l	
Mean ± SD	85.3±130.2
Median (range)	53.5 (9–925)
AST, U/l	
Mean ± SD	47.2±50.5
Median (range)	31 (13–357)
HBV DNA, IU/ml	
Mean ± SD	1.1×10 ⁷ ±2.7×10 ⁷
Median (range)	2.13×10 ⁴ (37 to 1×10 ⁸)
Inflammatory grade, n (%)	
0–1	60 (51.3)
2–4	57 (48.7)
Fibrosis stage, n (%)	
0–1	58 (49.6)
2–4	59 (50.4)

Table 2. Significant liver pathology by viral load and ALT level

Variable/levels	n (%)	≥F2	≥F2 and/or ≥A2
HBV DNA			
<2,000 IU/ml	23 (19.7)	8 (13.6)	12 (16.4)
2,000 to <20,000 IU/ml	32 (27.4)	12 (20.3)	14 (19.2)
≥20,000 IU/ml	62 (53.0)	39 (66.1) ³	47 (64.4) ⁵
ALT ¹			
≤1×ULN	54 (46.2)	15 (25.4) ⁴	22 (30.1) ⁴
>1× to <2×ULN	29 (24.8)	22 (37.3)	24 (32.9)
≥2×ULN	34 (29.1)	22 (37.3)	27 (37.0)
ALT ²			
≤1×ULN	18 (15.4)	3 (5.1)	7 (9.6)
>1× to <2×ULN	34 (29.1)	10 (16.9)	13 (17.8)
≥2×ULN	65 (55.6)	46 (78.0) ⁴	53 (72.6) ⁴
Total	117	59	73

Data expressed as n (%). ¹ Using the standard cutoff value of 40 U/l. ² Using the new cutoff values of 30/19 U/l. ³ p = 0.016. ⁴ p = 0.000. ⁵ p = 0.005.

Assessment of Liver Histopathology

All patients (n = 117) included in this study had ultrasound-guided liver biopsies (hematoxylin and eosin stains for morphological evaluation and Masson's trichrome stain for fibrosis assessment). Liver specimens were adequate in size (at least 15 mm long) and >6 portal tracts were assessed. All liver biopsy specimens were assessed and scored according to the METAVIR scoring system [16] by a single experienced hepato-histopathologist (H.A.) who was blinded to all clinical and virological results. Patients with inflammatory grade ≥A2 and/or those with fibrosis stage ≥F2 were considered to have significant pathology.

Statistical Analysis

Data were collected initially in a specialized data collection form and transferred to the Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, Ill., USA) for analysis. Means of continuous variables were compared using Student's t tests, nonparametric tests (Wilcoxon and Mann-Whitney), or one-way analysis of variance (ANOVA) with a post-hoc test (Tukey's) as appropriate. A χ^2 or Fisher's exact test was used to compare ratios and proportions. p < 0.05 was considered statistically significant. Correlation between continuous variables with skewed distribution was tested by Spearman's rank correlation.

Results

Baseline Patient Characteristics

We analyzed 117 patients with chronic HBV, HBeAg-negative, genotype D after exclusion of those who were HBeAg positive (n = 31) and those who were non-genotype D (n = 16). Of these, 97 (82.9%) male patients and 20 female patients, aged 14–70 years (median = 37), were included in the study. HBV DNA levels ranged from 32 to 1 × 10⁸ IU/ml (median = 2.13 × 10⁴) and the ALT level ranged from 9 to 925 U/l (median = 53.5), while AST ranged from 13 to 357 U/l (median = 31). Moderate-to-severe inflammation (≥A2) was detected in 57 (48.7%) patients, and significant fibrosis (≥F2) and significant pathology (≥F2 and/or ≥A2) were found in 59 (50.4%) and 73 (62.4%) patients, respectively (table 1).

Relation between HBV DNA and Liver Histopathology

According to the AASLD guidelines, 2007 standards, taking an HBV DNA level of 2,000 IU/ml as a cutoff value for HBeAg-negative patients, our results showed that 8 of 23 patients (34.8%) with a serum HBV DNA level <2,000 IU/ml had stage 2 or higher fibrosis (≥F2), and 12 of them (52.2%) had fibrosis ≥F2 and/or inflammation grade 2 or more (≥A2). Patients with HBV DNA >20,000 IU/ml showed significantly more hepatic pathology (≥F2) and (≥F2 and/or ≥A2) compared to patients with lower HBV DNA (p = 0.016 and 0.005, respectively; table 2).

Table 3. Comparison between the number and percentage of patients who need to be treated according to the strategies used: pathology, ALT, and HBV DNA levels

Question	Standard ALT cutoff value ¹ and HBV DNA (strategy 1)	New cutoff ALT values ² and HBV DNA (strategy 2)	≥F2 ± ≥A2 (strategy 3)	≥F2 and/or ≥A2 (strategy 4)
Decision to treat				
Candidate for therapy	31/117 (26.5)	58/117 (49.6)	59/117 (50.4)	73/117 (62.4)
Not a candidate	86/117 (73.5)	59/117 (50.4)	58 (49.6)	44 (37.6)
Cases missed				
Compared to strategy 2	27/117 (23.1)	–	–	–
Compared to strategy 3	28/117 (23.9)	1/117 (0.9)	–	–
Compared to strategy 4	42/117 (35.9)	15/117 (12.8)	14/117 (12.0)	–

Data expressed as n/n (%). ¹ ULN = 40 U/l. ² ULN = 19 U/l for females and 30 U/l for males.

Fig. 2. Number and percentage of patients that need to be treated. Decision 1: based on raised HBV DNA and standard ALT cutoff value (40 U/l) irrespective of pathology results. Decision 2: based on raised HBV DNA and new ALT cutoff value (19/30 U/l) irrespective of pathology results. Decision 3: based on presence significant fibrosis (≥F2 ± ≥A2). Decision 4: based on presence of and significant pathology (≥F2 and/or ≥A2).

Relation between ALT Level and Liver Histopathology

According to the AASLD guidelines, 2007 standards, using an ALT cutoff value of 40 U/l, our results showed that 15 of 54 patients (27.8%) with serum ALT levels ≤1 × ULN (40 U/l) had fibrosis stage ≥F2, and 22 of them (40.7%) had ≥F2 and/or ≥A2. However, patients with ALT ≤1 × ULN showed significantly less hepatic pathology: ≥F2 and ≥F2 and/or ≥A2 compared to those with higher ALT levels ($p = 0.000$ for both; table 2). Even with the new reduced ALT cutoff values, 3 of 18 patients (16.7%) with serum ALT levels ≤1 × ULN (19/30 U/l) had

stage ≥F2, and 7 of them (38.9%) had fibrosis ≥F2 and/or ≥A2 (table 2). Patients with ALT levels ≥2 × new ULN (19/30 U/l) had significantly more hepatic pathology: ≥F2 and ≥F2 and/or ≥A2 compared to those with lower ALT levels ($p = 0.000$ for both; table 2).

Candidacy to Treatment Decision

Based on serum HBV DNA (>2,000 IU/ml) and ALT levels >2 × 40 U/l (the standard cutoff value), only 31 (26.5%) patients were candidates for therapy. This increased to 58 (49.6%) patients when the new ALT cutoff values (30 U/l for males, and 19 U/l for females) were applied. For the start of therapy, implementation of significant fibrosis (≥F2 ± ≥A2) or significant liver pathology (≥F2 and/or ≥A2) increased patient eligibility for therapy to 59 (50.4%) and 73 (62.4%), respectively (table 3; fig. 2). This increased the candidacy for therapy by 1 (0.9%) and 15 (12.8%) patients, respectively. Furthermore, the application of the new lower ALT cutoff values and either ≥F2 ± ≥A2 and ≥F2 and/or ≥A2 further increased the candidacy for treatment by 28 (23.9%) and 42 (35.9%) patients, respectively, which would have been missed if we had only relied on strategy 1 (table 3).

Discussion

We believe that the current international guidelines that rely mainly on HBV DNA and ALT levels for the decision on how to treat patients with chronic HBV infection require regular reevaluation. Based on our results and those of others [17], liver histopathology, but not HBV genotype, needs to be incorporated more in future

practice guidelines. Currently available guidelines recommend observation of HBV-infected patients, especially those with viral load above the threshold and normal ALT level [10, 12, 13], whereas the Keeffe algorithm considers liver biopsy and treatment when biopsy reveals fibrosis [11], particularly in patients above the age of 40 years.

Recent reports revealed that 12–45.5% of patients with chronic HBV infection and persistently normal ALT levels have fibrosis stage $\geq F2$ [18–21], and that age >40 years is an independent predictor of significant pathology. This together with our results suggest that the future management guidelines of this patient group should recommend assessing hepatic pathology either by biopsy or by its alternative noninvasive modalities such as a FibroScan and/or FibroTest of hepatic fibrosis.

Our patients had HBeAg-negative disease, which usually needs closer follow-up and frequent monitoring [22]. This requires not only extra costs, but also patient awareness and education, as most of these patients are asymptomatic. In addition, many of these patients present at an older age with significant hepatic pathology and persistent viral replication [7–9]. In addition, our patients have HBV genotype D, the predominant type in Saudi Arabia and other Middle East countries like Egypt [3, 23]. Unfortunately, this genotype is known to be associated with the HBeAg-negative variant [6], and with more severe liver disease [7]. Therefore, patients with HBV infection, especially those with genotype D and the HBeAg-negative variant, may need earlier liver biopsy rather than frequent laboratory follow-up with ALT and HBV DNA evaluations, which are often fluctuating, particularly HBV DNA levels [24]. This view is supported by the Keeffe algorithm, which considers therapy in patients with known significant histological disease even if ALT is within the normal range and HBV DNA is low [11].

In this study, 50% of HBeAg-negative patients exhibited significant liver pathology and were associated with normal or slightly elevated ALT level (table 2). This supports the concept that lower HBV DNA levels ($3\text{--}5 \log_{10}$ IU/ml) may be associated with progressive liver disease and therefore warrants treatment [13].

The more recent guidelines recommend reducing the ULN ALT and AST to 30 U/l for men and 19 U/l for women [11, 13]. Applying these new values in the present study increased the eligible patients for treatment by 23.1% (27 patients; table 3). We have also shown more patients with significant fibrosis ($\geq F2 \pm \geq A2$) and significant pathology ($\geq F2$ and/or $\geq A2$) in patients with higher HBV DNA levels (61.1 and 64.4% respectively; table 3). This is consistent with previous reports in HBeAg-negative patients [17, 25, 26]. These results are not universal as HBV DNA load and ALT levels failed to predict disease activity in other studies [27–29].

Detection of significant liver pathology ($\geq F2$ and/or $\geq A2$), or significant fibrosis ($\geq F2 \pm \geq A2$) increased the number of cases eligible for treatment to 59 (50.4%) and 73 (62.4%), respectively. This increased the candidacy to therapy by 42 (35.9%) and 28 (23.9%) patients, respectively. Despite the increased use of transient elastography (FibroScan) in the assessment of hepatic fibrosis, liver biopsy is still the gold standard due to the discordance of the available literature [30, 31].

Liver biopsy remains an important tool in the diagnosis and management of parenchymal liver diseases. However, it remains an invasive procedure with certain unavoidable risks and complications as well as shortcomings in diagnostic accuracy, as a large liver sample is required for an ideal assessment. It is also open to observer interpretation and prone to sampling errors [32]. In recent years, a number of noninvasive biomarkers with an impressive range of diagnostic certainty approaching that achieved with liver biopsies have evolved [33]. These noninvasive tests represent a lower-cost option, are easily reproducible, and serve as suitable alternatives to assess hepatic fibrosis.

In conclusion, liver biopsy is more reliable than both ALT and HBV DNA levels in the decision to treat patients with HBeAg-negative chronic HBV genotype D, even with implementation of the recommended lower ALT levels. Whether to replace liver biopsy with the currently evolving noninvasive measure is a debatable issue that warrants future investigations.

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