Brief Original Articles

Rethinking therapeutic decisions for hepatitis B infection in Syria: insights into molecular monitoring

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Abstract

Introduction: Hepatitis B virus patients are usually treated in Syria with alpha interferon and nucleos(t)ide analogues. Genotypic viral factors causing inadequate response or relapse following initial response are not routinely investigated. This study aimed to explore and discuss local therapeutic decisions from a molecular perspective.

Methodology: Fifty patients with hepatitis B from Syria were tested for HBV genotyping and drug-resistance mutations by DNA sequencing.

Results: All patients had genotype D, which is characterized by relatively low response to interferon-based therapy. Drug-resistant viral mutant variants were detected in one fifth of the enrolled patients, and distributed similarly in both nucleos(t)ide analogues-naïve and -treated patients. However, nucleos(t)ide analogues-based therapy was associated with the existence of more mutations and hence increased resistance.

Conclusions: Investigating HBV genotypes and drug-resistance mutations to support treatment decisions is critically needed for efficient therapy and patients' survival.

Key words: hepatitis B virus; Syria; therapeutic decisions; viral genotype; mutant variants; drug resistance

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Introduction

Hepatitis B virus (HBV) infection is a highly prevalent disease worldwide and considered a major public health problem [1]. Chronically infected patients are at high risk of cirrhosis and hepatocellular carcinoma (HCC) [2], while recommended management is limited to the treatment of its complications and antiviral therapy [3]. HBV demonstrates a high mutation rate due to its error-prone reverse transcriptase that lacks exonuclease proofreading activity [4]. Hence drug-resistant variants may emerge throughout nucleos(t)ide analogues-based therapy leading to relapse and hepatic decompensation [5,6]. Moreover, the HBV genotype plays an essential role in driving viral evolution [5] as well as response to interferon-based therapy [7,8].

In Syria, hepatitis B patients are treated with either interferon alpha (INF-α) or nucleos(t)ide analogues, such as Lamivudine (LMV) and Adefovir (ADV). Because they are the only drugs approved by the national drug committee, they are solely recommended by the national guidelines for hepatitis B management considering INF-α as the first-line therapy [9]. Currently, therapeutic decisions are based on viral DNA load in patients' sera which is inadequately informative as to the reason behind frequently encountered cases of relapse or inadequate response to treatment. These cases remain unexplained and problematic to manage appropriately. This study aimed to explore the appropriateness of local therapeutic decisions in view of the genotypic viral factors influencing response to therapy in a group of hepatitis B patients with high viral loads.

Methodology

Serum samples from fifty Syrian hepatitis B patients with high viral loads were drawn between August 2008 and April 2010 after obtaining informed consent. Sera viral loads were more than 6 log with an average of 1.45E+9 copies/ml (1.83E+6-1.98E+10 copies/ml) in both treatment-naïve (26 of 50, 52%) and treated (24 of 50, 48%) patients when enrolled in the study. Information on applied therapeutic plans was obtained from the patients' medical records. Twenty-four patients were treated with either interferon monotherapy (9 of 50, 18%) for 8 months (3-18 months), or interferon and nucleos(t)ide

Table. Nucleos(t)ide analogues resistance mutations detected in Syrian hepatitis B patients (n = 50)[a]

Patient	Administered Drug[d]	Detected Mutation	Associated Resistance[d]
Nucleos(t)ide analogues-treated patients (n=15)[b]			
1	LMV	rtL180M	LMV-resistance [3]
		rtM204I	LMV-resistance [3]
2	LMV	rtL80V	LMV- & ADV-resistance [11]
		rtL180M	LMV-resistance [3]
		rtM204V	LMV-resistance [3]
3	LMV/ADV	rtL80I	LMV- & ADV-resistance [11]
		rtV84M	ADV-resistance [11]
		rtL180M	LMV-resistance [3]
		rtM204I	LMV-resistance [3]
4	LMV/ADV	rtS135Y	LMV-resistance [5]
Nucleos(t)ide analogues-naïve patients (n=35)[c]			
5	NA	rtS135Y	LMV-resistance [5]
6	NA	rtS135Y	LMV-resistance [5]
7	NA	rtQ215S	LMV- & ADV-resistance [11]
8	NA	rtQ215S	LMV- & ADV-resistance [11]
9	NA	rtV214A	ADV-resistance [11]

[a] Viral mutant variants similarly existed in both nucleos(t)ide analogue-naïve and -treated hepatitis B patients [$\chi^2(1, N=50) = 1.09$, p=.29]. Number of resistance mutations was significantly higher in nucleos(t)ide analogue-treated patients [$t(7) = 2.65$, $p<.05$].
[b] Patients were treated with nucleos(t)ide analogue monotherapies for 27 months (6-72 months).
[c] Patients were either treatment-naïve (26 of 35) or treated with interferon monotherapy (9 of 35) for 8 months (3-18 months).
[d] LMV, Lamivudine; ADV, Adefovir; LMV/ADV, LMV & ADV sequential monotherapies; NA, non-applicable.

analogues sequential monotherapies (15 of 50, 30%) for 27 months (6-72 months).

Viral DNA was extracted using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany). The entire viral pre-S1/pre-S2/S gene region was amplified in a 50 µl-volume reaction containing 1 µM of each primer (forward, 5'-GGG TCA CCW TAT WCY TGG GAA-3'; reverse, 5'-CGT TGC CKR GCA ACS GGG TAA AGG-3'; TIB MOLBIOL, Berlin, Germany), 1.5 mM of MgCl2 and 5 µl of extracted DNA using a GeneAmp High Fidelity PCR System (Applied Biosystems, Foster City, CA, USA). Thermal cycling profile was initiated at 94°C for 2 minutes followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds with an extension at 72°C for 3 minutes. An additional extension time of 5 seconds per cycle after cycle 10 and a final extension step at 72°C for 7 minutes were added. Direct sequencing was performed on an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using previously described primers [10,11]. Viral nucleotide sequences were subtyped using the NCBI Genotyping tool and Bayesian phylogenetic analysis. Predicted protein translations were searched for thirty mutations previously reported as associated with viral resistance against various nucleos(t)ide analogues including rtL80V/I, rtV84M, rtS85A, rtS135Y, rtI169T, rtV173L, rtL180M/C, rtA181T/V/S, rtT184G/A/S, rtA194T, rtS202I/G, rtM204V/I/S, rtV214A, rtQ215S, rtL229V, rtN236T, rtP237H, rtN238T/D and rtM250V/L [3,5,12]. Chi square and Student's t tests were used to analyze mutation distribution among nucleos(t)ide analogues-naïve and -treated patients. Viral nucleotide sequences related to our study were assigned GenBank accession numbers from JN257148 to JN257217.

Results

Genotype D was assigned to all obtained viral nucleotide sequences. LMV- and/or ADV-resistant viral mutant variants were detected in nine patients (9 of 50, 18%). Up to 4 mutations of rtL80V/I, rtV84M, rtS135Y, rtL180M and/or rtM204V/I were detected in 4 patients (4 of 15, 27%) treated with nucleos(t)ide analogues, while one mutation of rtS135Y, rtV214A or rtQ215S was detected in 5 recently diagnosed treatment-naïve patients (5 of 35, 14%) [Table].

Viral mutant variants similarly existed in both nucleos(t)ide analogue-naïve and -treated hepatitis B patients [$\chi2(1, N = 50) = 1.09$, $p = .29$]. However, the number of resistance mutations was significantly higher in nucleos(t)ide analogue-treated patients [$t(7) = 2.65$, $p < .05$].

Discussion

Our findings indicate the dominance of genotype D in Syria, which is consistent with its high prevalence

in the Mediterranean region [2,13]. Despite the reported association of genotype D with poor response to interferon-based therapeutic regimens [7,8,14], interferon alpha is routinely prescribed as the first-line therapy to hepatitis B patients in Syria [9]. Interestingly, altering the therapeutic plan from interferon alpha to nucleos(t)ide analogues monotherapies had already been indicated by physicians for two thirds (15 of 24, 63%) of the treated patients, inferring the ineffectiveness of interferon-based therapy, as shown in the present study. Selecting effective therapeutic plans is critically needed due to the reported association of genotype D with high risk of severe liver disease, recurrence, and fulminant hepatitis [2,7]. Fortunately, genotype D influence on driving viral evolution leading to the emergence of resistance mutations under nucleos(t)ide analogues pressure is relatively low [5,14]. Thus considering HBV genotype is crucial for therapeutic decision making whether by interferon or nucleos(t)ide analogues [8].

Viral mutant variants similarly existed in both nucleos(t)ide analogue-naïve and -treated hepatitis B patients. Hence investigating resistance mutations beforehand is recommended for efficient drug prescription. Moreover, monitoring drug-resistant mutants during nucleos(t)ide analogues treatment is necessary to ensure early detection of potentially emerging mutations before considerable serum viral load increase in the patient occurs [3,5,15]. This necessity is underscored by our findings of detecting multiple resistance mutations in LMV/ADV-treated patients whose therapeutic plans were continued due to lack of awareness of the critical necessity of timely rescue therapy, and hence were at high risk of mutant selection that led to viral breakthrough, and might lead to hepatitis flare, hepatic decompensation and death [3,15].

In conclusion, the critical need for evidence-based therapeutic decisions urges review of local established therapeutic regimens. National guidelines should be updated taking into account the dominance of genotype D in Syria and considering more efficacious nucleos(t)ide analogues with high barriers to resistance, such as Entecavir and Tenofovir [16]
Additional molecular investigations, such as HBV genotypes and drug-resistant mutations, should also be considered for efficient therapies and patients' survival.

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