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Short Communication

HBsAg kinetics after 7 years of therapy with tenofovir disoproxil fumarate in a cohort of naïve patients affected by chronic hepatitis B with different genotypes

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ABSTRACT

The role of different genotypes in nucleos(t)ide analogs (NAs) treatment is still debated. Previous studies conducted on special populations evidenced that the E genotype had the lower virological and serological response. This descriptive study aims to recognize the hepatitis B "s" antigen (HBsAg) decline during tenofovir disoproxil fumarate (TDF) treatment in a cohort of patient affected by chronic hepatitis B (CHB). We retrospectively included all patients with CHB treated with TDF between April 2007 and March 2012 with a duration of treatment of 7 years. Kinetics of HBsAg was determined as serological response in this cohort. We include 110 subjects; virological response was observed in all subjects with genotypes A, B, and D; in 17 patients with C genotype (94.4%) and 24 with E genotype (96%). HBeAg loss was observed in 2 patients with genotype A (50%), 3 with B (100%), 0 with C (0%), 1 with D (20%), and 1 with E genotype (25%). In multivariate analysis we observed as predictive factors of HBsAg decline the baseline level of HBsAg (OR = 1.467; 95%CI: 1.221–5.113; $p = 0.017$) and viral genotypes (OR = 11.218; 95%CI: 5.441–41.138; $p < 0.001$). This study confirmed higher HBsAg decline after 7 years of treatment in A and B genotypes, and lower in C, E, and D genotypes. However, no evidence is enough to choose a single NAs, but in special populations, as well as in genotype E, the use of TDF should be preferred to entecavir.

1. Introduction

Chronic infection caused by hepatitis B virus (HBV) is a relevant global health problem, leading to chronic hepatitis B (CHB) with different clinical involvement: liver inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1].

The goals of treatment are different according to epidemiological, clinical, and virological characteristics of infection; the most desirable outcome should be the hepatitis B surface antigen (HBsAg) loss with or without seroconversion, but realistic targets are more often the virological or biochemical response with HBV-DNA long-term suppression and consequent liver function test normalization. Different approaches are finite-course of treatment using pegylated interferon alpha (PEG-IFN) or indefinite course with nucleoside analogs (entecavir, ETV) or nucleotides analogs (tenofovir disoproxil fumarate, TDF; tenofovir alafenamide fumarate, TAF). The treatment with PEG-IFN may lead to a 1% to 4% of serological response at 48 weeks but is affected by several side effects, and the effectiveness declines after treatment stop-

Abbreviations: HBV, Hepatitis B virus; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; qHBsAg, quantitative hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibody to hepatitis B e antigen; anti-HBs, antibody to hepatitis B surface antigen; NAs, nucleos(t)ides analogues; ETV, entecavir; TDF, tenofovir disoproxil fumarate; IFN, interferon; PEG-INF, pegylated interferon.

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ping; conversely, indefinite therapy with nucleos(t)ides analogs (NAs) is well tolerated but seroconversion rate is very low [2].

Several studies evidenced the role of different HBV genotypes in the response to treatment both with PEG-IFN [3–7] than NAs [8,9], and the data showed a better response in genotypes A, B, and D than C and E [10].

In this retrospective study, we evaluated the HBsAg decline during the treatment with TDF in a cohort of patients affected by CHB and different viral genotypes after 7 years of therapy.

2. Patients and methods

2.1. Patient population

We retrospectively evaluated patients affected by CHB and treated with TDF in the Centre of Infectious Diseases "Amedeo di Savoia Hospital". Inclusion criteria were: adult age (>18 years); chronic hepatitis B with a documented HBsAg- positive for at least 6 months; baseline HBV-DNA level > 2000 IU/mL and ALT > 40 IU/mL; HBeAg-negative/antiHBe-positive, naive for previous treatment with IFN or other NAs. Exclusion criteria were: co-infection with hepatitis C or D virus or the human immunodeficiency virus (HIV).

Median follow-up time from treatment starting was 7 years. Patients lost at follow-up or with treatment interruption (virological failure, death, or toxicity) were not included in this analysis.

2.2. Study end points

Primary endpoint of the study was the description of HBsAg kinetics after 7 years of treatment with TDF in a cohort of CHB naïve patients. Secondary endpoint was the evaluation of HBsAg kinetic among different viral genotypes.

2.3. Assays

Serum HBV-DNA levels were quantified by the Real Time PCR COBAS AmpliPrep/COBAS TaqMan HBV Test 2.0 (Roche Molecular Systems). HBV genotypes were determined using the INNOLIPA reverse hybridization assay (Innogenetics). HBsAg, HBeAg and anti-HBe were detected by the Elecsys instrumental platform (Roche Diagnostics); quantitative HBsAg test (qHBsAg) was performed with ARCHITECT HBsAg (Abbott Diagnostics) with a dynamic range of 0.05–250.0 IU/mL; qHBsAg values above 250.0 IU/mL were subsequently 1:100 serially diluted and retested until falling within the dynamic range. Liver fibrosis stage was expressed in kPa using the Fibroscan. According to the "European Association for the Study of the Liver" (EASL) guidelines, the qHBsAg measurement was performed every six months after treatment starting. Virological response is defined as undetectable HBV DNA by a sensitive polymerase chain reaction (PCR) assay with a limit of detection of 10 IU/mL [2].

2.4. Statistical analysis

For descriptive statistics, continuous variables were summarized as median and inter-quartile ranges (IQR: 25th–75th percentiles) and ranges. Categorical variables were described as frequencies and percentages. All data were tested for normality using a Shapiro-Wilk test. Differences in categorical data between groups were analyzed using Kruskal-Wallis and Mann-Whitney tests. To investigate continuous data, a Spearman Rank correlation was used. The association was calculated using the χ^2-test. Univariate and multivariate analyses were performed using a logistic regression considering the main clinical and virological variables. Statistical analyses were conducted by SPSS software package ver. 29.0.

3. Results

3.1. Baseline characteristics of the population

We considered in this analysis 110 subjects who started treatment with TDF between April 2007 and March 2012 with a follow-up of at least 7 years. Clinical and virological characteristics of the study population were reported in the Table 1. Significant differences among viral genotypes were reported for age, risk factors, geographical origin, and male sex. HBeAg-positive patients were: 4 with the A genotype (19%), 3 with the B genotype (20%), 4 in the C genotype (22.2%), 5 with the D genotype (16.2%), and 4 in the E genotype (16%).

3.2. Outcomes of treatment at 7 years of follow-up

Virological response was observed in all subjects with genotypes A, B, and D; in 17 patients with C genotype (94.4%) and 24 with E genotype (96%). HBeAg loss was observed in 2 patients with genotype A (50%), 3 with B (100%), 0 with C (0%), 1 with D (20%), and 1 with E genotype (25%). HBsAg loss was observed in 2 patients with A genotype (9.5%) and 1 with seroconversion (4.8%); in 3 patients with B genotype (20%) and 4 with seroconversion (26.7%); no serological response was observed in other genotypes (Table 1).

3.3. Serological HBsAg decline after 7 years of therapy

After 7 years of therapy, we observed the following median HBsAg declines: 2.2 log IU/mL in genotype A (IQR: 1.9–2.8); 3.3 log IU/mL in genotype B (IQR: 3.2–4.1); 0.9 log IU/mL in genotype C (IQR: 0.8–1.1); 2.1 log IU/mL in genotype D (IQR: 1.6–2.7); 1.8 log IU/mL in genotype E

Table 1
Baseline characteristics and clinical outcomes of study population.

Characteristics	VIRAL GENOTYPES (N = 110)					*p value*
	A	B	C	D	E	
Number of patients *n* (%)	21	15	18	31	25	ns
Age (yr) median [IQR]	46 [31–62]	43 [33–64]	41 [30–58]	72 [61–86]	28.5 [21–50]	<0.001[a]
Risk factors *n* (%)						
Intravenous drug use	5 (23.8)	0 (0)	1 (5.5)	8 (25.8)	0 (0)	<0.001[b]
Transfusion	4 (19)	1 (6.7)	3 (16.7)	11 (35.5)	0 (0)	<0.001[c]
Sexual	6 (28.6)	3 (20)	4 (22.2)	6 (19.3)	4 (16)	ns
Family history of HBV	1 (4.8)	6 (40)	6 (33.3)	1 (3.2)	12 (48)	<0.001[d]
Unknown	5 (23.8)	5 (33.3)	4 (22.2)	5 (16.1)	9 (36)	0.012
Male sex *n* (%)	14 (66.7)	9 (60)	12 (66.7)	16 (51.6)	20 (80)	<0.001[e]
Geographical origin *n* (%)	Italy: 5 (23.8) East-Europe: 15 (71.4) Africa: 1 (4.8)	China: 15 (100)	China: 18 (100)	Italy: 25 (80.6) Africa: 4 (12.9) East-Europe: 2 (6.4)	Africa: 25 (100)	<0.001[f]
HBeAg positive *n* (%)	4 (19)	3 (20)	4 (22.2)	5 (16.2)	4 (16)	ns
Liver stiffness (kPa) median [IQR]	8.9 [7.4–12.7]	8.2 [7.3–11.2]	8.6 [7.3–12.7]	9.2 [7.5–12.4]	8.5 [7.2–10.4]	ns
qHBsAg (log IU/mL) median [IQR]	3.2 [2.7–3.7]	3.4 [2.4–3.9]	3.6 [2.5–3.9]	3.6 [2.7–3.8]	3.4 [2.1–3.9]	ns
HBV-DNA (log IU/mL) median [IQR]	4.3 [3.6–5.2]	4.8 [3.2–5.1]	4.4 [3.9–5.1]	4.4 [3.1–5.2]	4.5 [3.1–5.6]	ns
ALT (IU/L) median [IQR]	66 [54–111]	70 [59–132]	74 [56–155]	64 [50–104]	71 [56–128]	ns
Clinical outcomes at 7 years of treatment *n* (%)						
Virological response	21 (100)	15 (100)	17 (94.4)	31 (100)	24 (96)	ns
HBeAg loss	2 (50)	3 (100)	0 (0)	1 (20)	1 (25)	<0.001[g]
HBsAg loss	2 (9.5)	3 (20)	0 (0)	0 (0)	0 (0)	<0.001[h]
HBsAg loss and anti-HBs +	1 (4.8)	4 (26.7)	0 (0)	0 (0)	0 (0)	<0.001[i]

Note: ns, non significant. [a] Genotypes D and E vs others; [b] Genotype D vs others; [c] Genotype D vs others; [d] Genotype E vs others; [e] Genotype E vs others; [f] Genotypes C, B, E vs others; [g] Genotypes A and B vs others; [h] Genotypes A and B vs others; [i] Genotypes A and B vs others.

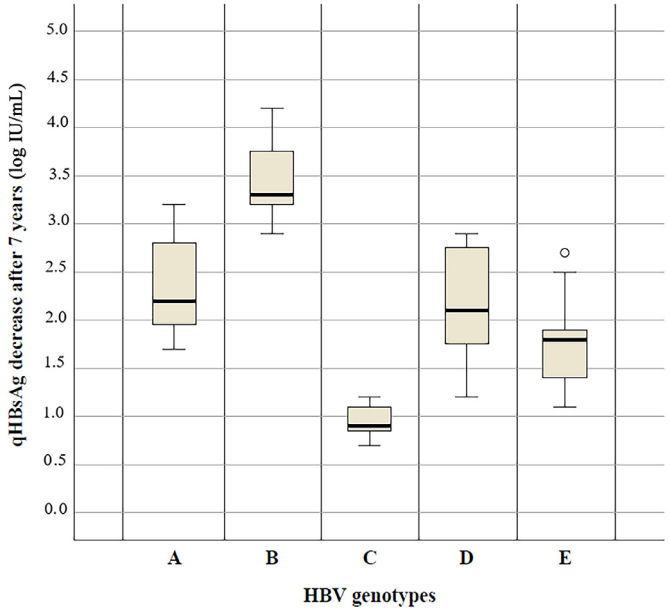

Fig. 1. Different HBsAg decline during the treatment with TDF in the study population according to viral HBV genotypes.

(IQR: 1.3–1.9). Median declines were significant different in genotype A vs B, C, and E ($p < 0.001$); genotype B vs all the others ($p < 0.001$); genotype C vs all the others ($p < 0.001$); genotype D vs B, C and E ($p < 0.001$); genotype E vs A, B and C ($p < 0.001$) (Fig. 1).

3.4. Univariate and multivariate analysis

The following factors are considered in univariate analysis considering the HBsAg decline: age, male sex, HBeAg-positive status, HBV-DNA level, ALT, qHBsAg, liver stiffness, and viral genotypes. Baseline level of HBsAg (OR = 1.224; 95%CI: 1.127–4.289; $p = 0.021$) and viral genotypes (A and B vs others) (OR = 9.617; 95%CI: 3.516–32.943; $p < 0.001$) resulted significantly associated with HBsAg decline at 7 years of treatment. In multivariate analysis, we observed as predictive factors of HBsAg decline the baseline level of HBsAg (OR = 1.467; 95%CI: 1.221–5.113; $p = 0.017$) and viral genotypes (OR = 11.218; 95%CI: 5.441–41.138; $p < 0.001$) (Table 2).

Table 2
Univariate and multivariate analysis considering factors associated with qHBsAg decline in the study population.

Univariate analysis			
Factors	OR	95%CI	*p* value
Age	0.481	0.161–2.993	0.561
Male sex	0.561	0.224–4.918	0.443
HBeAg-positive status	1.221	0.898–7.476	0.339
HBV-DNA (log IU/mL)	1.556	0.784–9.561	0.417
ALT (IU/mL)	0.892	0.455–4.213	0.617
qHBsAg (log IU/mL)	1.224	1.127–4.289	0.021
Liver stiffness (kPa)	0.782	0.335–4.432	0.617
Viral genotypes[a]	9.617	3.516–32.943	<0.001
Multivariate analysis			
qHBsAg (log IU/mL)	1.467	1.221–5.113	0.017
Viral genotypes[a]	11.218	5.441–41.138	<0.001

[a] Genotypes A and B vs others.

4. Discussion

Currently, at least 10 different HBV genotypes have been reported (A–J) with variable DNA sequence divergence from 4% to 8% [11]. Viral genotypes are strongly related to the geographical origin of patients, course of

illness, rate of seroconversion, response to PEG-IFN, and antiviral therapies [12–14]. In our previous study, we reported different kinetics of HBsAg decline in genotype D according to NAs [10], we observed also an overall low virological and serological response to PEG-IFN and NAs in the viral E genotype; in particular, this genotype seems to respond better to therapy with TDF than entecavir [9]. However, based on the latest data from this study, genotype C showed the worst virological response to TDF, followed by E and D genotypes. The greater HBsAg decline was observed in A and B genotypes, as already reported about PEG-IFN treatment. Despite the viral genotype being considered a relevant choice factor in the PEG-IFN treatment, no current evidence is available about the role of the HBV genotype in the NA choice. Due to the cumulative rate of viral resistance, the recommended antiviral are entecavir and TDF [2], but there is not a criteria for choosing entecavir instead of TDF. We excluded the lack of treatment adherence mainly using the self-reported evaluation, with a rate of 95% to 98% adherence. This study has mainly a descriptive role; however, as previously described, the choice of NAs can be done by taking into consideration special populations such as young immigrant patients with genotype E [9] wherein the optimal choice should be the TDF treatment.

Due to a low number of patients enrolled in this study, the conclusion is not definitive and reliable results should be confirmed by other analysis with a large number of patients.

In conclusion, this study confirmed higher HBsAg decline kinetics after 7 years of treatment in A and B genotypes, and lower in C, E, and D genotypes. No stronger evidence is enough to choose a single NAs, but in special populations, as well as young patients with genotype E TDF therapy seems to lead to a better HBsAg decline and virological response.

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Author contributions

L.B.: conceptualization, data curation, and writing original draft. G.S., T.L., V.D. and G.D.P.: paper revision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

The data that support the findings of this study are available upon reasonable request.

Ethics statement

This study was approved by the local ethics committee as “HBV-Analogues Study” (Prot. N°002360; 26/1/2015) and all included subjects provided written informed consent.

Informed consent

Written informed consent was obtained from the patients for publication of this manuscript and any accompanying images.

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