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HBs ANTIGEN TITER DYNAMICS AS A PREDICTIVE FACTOR FOR TREATMENT WITH NUCLEOSIDE ANALOGUES IN PATIENTS WITH CHRONIC VIRAL HEPATITIS B

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In patients with chronic hepatitis B the viral DNA is the most important prognostic factor, which determines disease activity, efficiency of the therapy, and the risk of complications like liver cirrhosis and hepatocellular carcinoma (HCC). Since nucleoside analogues (NUCs) clear HBV DNA in a relative short time but don't achieve seroconversion in HBs system we need to find other ways to assess the virological status of patients that are in therapy with NUCs. Quantitative HBsAg (surface antigen), is the best marker that shows the amount and activity of cccDNA (covalently closed circular DNA) within hepatocytes. The aims of these study were to identify the patients that would respond best to nucleoside analogues treatment, better assessment of long term treatment and to determine in which patients and at what time we should change the treatment regimen. We monitored the dynamics of HBs quantitative antigen in 131 patients with chronic hepatitis B, 56 of them receiving Entecavir and 75 Tenofovir. Our endpoints were HVB sustained response, correlations between the rate of HBs antigen decrease and HBV DNA clearance, HBe seroconversion (in HBe positive patients), HBs seroconversion. We included 131 patients, 44 female and 87 male, within an age range of 18 to 74, median age 45, 56 under treatment with Entecavir and 75 with Tenofovir, 89 HBe positive and 42 HBe negative. Median duration of observation was 96 weeks. We correlated the dynamics of HBs antigen titer under treatment with viral AND clearance rate, seroconversion in HBe system and seroconversion in HBs system. We had 130 patients who cleared VHB DNA, 71 seroconversions in HBe system, one HBs seroconversion. The HBs antigen titers decreased the most in HBe positive patients who achieved HBe seroconversion, in HBe negative patients the decrease was significantly lower. The decrease in quantitative HBsAg was very slow suggesting that NUCs offer a very low rate of HBs seroconversion and in a very long time. NUCs are efficient in HVB DNA clearance, most patients achieved undetectable DNA in the first three months of therapy. The best results of NUCs on HBs antigen titers were on HBe positive patients who achived seroconversion. We had a patient who achieved HBs seroconversion in 48 months, this was a HBe positive patient with low baseline levels of HBs antigen.In HBe negative patients NUCs were less effective on HBs antigen, the decrease was less significant than in HBe positive patients. We had a patient that developed Entecavir resistence, this fact was preceded by an increase of the HBs antigen levels while under treatment.

Key words: HBsAg, HVB DNA, HBeAg, Seroconversion, Viral clearance

INTRODUCTION

HBV infection represents a major health problem worldwide. It can cause both acute and chronic liver disease. It is estimated that over 240 million people are chronically infected. More than 780 000 people die every year because of its complications: cirrhosis and hepatocellular carcinoma. Although major breakthrough has been made regarding prevention and treatment of HBV infection, the disease remains an important health issue for healthcare workers[1]. Chronic hepatitis B is defined as a positive hepatitis B surface antigen (HBsAg) for more than 6 months.

HBV infection has a non-linear evolution. Three phases have been identified as part of the natural course of the disease: immune-tolerant with high HBV-DNA, normal alanin-amino-transferase (ALT) levels, positive HBeAg and minimal liver damage; an immune active phase with high HBV-DNA, elevated ALT levels, positive HBeAg (patients may develop anti-HBe), active liver inflammation and an inactive phase with HBV-DNA levels of <2000IU/mL or even undetectable viral load, normal ALT level, negative HBeAg and positive anti-HBe with minimal inflammation and fibrosis on liver histology[2,3].

Although HBV infection can be prevented through vaccination, antiviral treatment is also of great importance in reducing the morbidity and mortality caused by its complications. Currently there are seven antiviral agents approved for the treatment of chronic hepatitis B: Lamivudine, Adefovir, Entecavir, Telbivudine, Tenofovir, Emtricitabine, standard and PEG-IFN[1]. The ultimate goal of these therapies is HBsAg clearance. Nevertheless in most people the treatment does not cure hepatitis B, only suppresses the viral replication which is also important as long term survival is improved and progression to cirrhosis and liver cancer are slowed. It has been shown that Entecavir and Tenofovir are the most potent antiviral agents primarily in inducing undetectable HBV-DNA load and also in improving liver histology and decrease of ALT levels[4] However, these treatments only suppress the viral load and have a low rate in curing hepatitis B, as stated before.

HBV-DNA levels have been used for monitoring treatment response. However these molecular techniques by which the viral load is determined are expensive and not always accessible.

A more accessible way of predicting treatment outcome which has been studied in past years has been quantification of HBsAg[5.]

Studies have shown that quantitative HBsAg varies during treatment with interferon and nucleoside analogues. QHBsAg decreases more rapidly during interferon treatment and it seems it is associated with a higher chance of sustained virological response (SVR). Conversely, a much slower decrease has been observed during treatment with nucleoside analogues, which diminishes its ability of being used as a treatment response predictor during the first years of nucleotide analogue therapy. [6,7 8]

However studies conducted on patients receiving nucleoside analogues showed that there is a correlation between HBsAg decrease and viral response, but it applies mostly in those who achieved seroconversion in the HBe system.[9,10].The aims of the study were to identify the patients that would respond best to nucleoside analogues treatment.

MATERIAL AND METHODS

The patients in our study were selected from the HBV infected patients treated in our clinic in the 1th janauary 2011- 31 december 2015 interval. We included chronic hepatitis B patients comprising both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients. The patients had a long term follow up in our unit and were assessed every 3 to 6 months. We excluded patients with hepatitis C virus or hepatitis D virus co-infection, alcoholic or with an autoimmune liver disease, or human immunodeficiency virus infection. The study group was comprised of 131 HBV patients who undergone treatment with nucleoside analogues. 66,41 % were men and 33,51% were women, 57,25% received Tenofovir and 42,75% Entecavir, 67,93% were HBeAg positive and 32,06% were HBeAg negative. The median follow-up period was 96 weeks.

Laboratory Measurements

The upper limit of normal ALT was set by the clinical laboratory at 36 U/L for both men and women.

Serum HBsAg levels were quantified retrospectively using the Synevo Electro-chemiluminescence immunoassay (ECLIA), (detection limit, 0.05 IU/mL; Synevo, Bucharest, Romania) according to the manufacturer. Serum HBV DNA levels were assessed using RT-PCR, HBV monitor test (lowest detection limit, 20 IU/mL; Synevo, Bucharest, Romania).

Concerning viral genotype we were able to determine it only in 12 patients (9,16%) due to economical consideration. We had 9 genotype D patients and 3 genotype A.

Statistical Analysis The continuous variables were presented as the median (range) and compared between patients. The categoric variables were analyzed using the chi-square test or the Fisher exact test. The serum HBV DNA level below detection limit was considered as 20 IU/mL in calculations. Statistical procedures were performed with SPSS software version 21 for Windows. A P value of less than .05 was considered statistically significant. This study was conducted with the approval of the Fundeni Clinical Institute. All patients signed a written consent before inclusion into the study and the study was approved by the Ethical Committee.

RESULTS AND DISCUSSIONS

Dynamics of quantitative Hepatitis B Surface Antigen and Hepatitis B Virus DNA. The patient characteristics and results of HBsAg and HBV DNA level assessment of the HBe positive and HBe negative are compared in Table 1. The median baseline HBsAg levels in the study group were 6905 IU/mL (56,249847). Serum HBV DNA median levels were 2608 UI/mL (20,53384510) (Table 1).

In the patients with HBeAg-positive, qHBsAg correlated satisfactory with serum HBV DNA while in the patients with HBeAg-negative, qHBsAg correlated poorly with serum HBV DNA.

During the first six months of therapy 130 patients (99,23%) achieved undetectable HBV DNA p>.001, one patient

(0,77%) developed resistance to Entecavir (Figure 1). The particularity of the patient that developed resistance to Entecavir was the fact that he underwent Lamivudine treatment three years before starting the treatment with Entecavir.

Variable	Value 1	Value 2
Patient n	131	
Age y	Median 45	(17-74)
Sex	Male 87	Female 44
Hbs level (median)	6905 UI/mL	
Hbe status	Positive 89	Negative 42
HVB DNA (median)	2608 UI/mL	
Treatment	Entecavir 56	Tenofovir 75

Table 1. Baseline Characteristics of Patients in our study group.

Concerning the HBsAg levels the decrease was much slower, the mean decrease in all the 131 patients was 0,27 log10 p>.01. (Figure 2) There were also patients in which the HBsAg levels increased, in 17 patients (12,97%) we observed an increase in HBsAg levels despite the significant decrease in HBV DNA. After 96 weeks of treatment 2 patients (1,52%) achieved HBsAg clearance, one of them (0,76%) developed HBs seroconversion. We also had one patient who developed HBs antibodies while still having positive HBs antigen, the reason underlying the presence of both HBs Ag and anti-HBs is unknown - one hypothesis might be the selection of immune variants of HBsAg.
The rate of HBe seroconversion was high, 71 (79,77%) of the 89 patients achieved seroconversion. The patients who achieved HBe seroconversion also had the best results concerning qHBs decrease.

Table 2. Summary of results after 96 weeks of treatment with nucleosidic analogs (Entecavir or Tenofovir).

Variable	Value	Procent
Median HBs decrease	0,27 log 10.	
HVB DNA clearence	130 out of 131	99,23%
HBs antigen clearance	2	1,52%
HBs seroconversions	1	0,76%
HBe seroconversions	71	79,77%
Increase in HBs titers	17	12,97%
Resistance to treatment	1	0,76%
Coexisting HBs antigen and antibody	1	0,76%

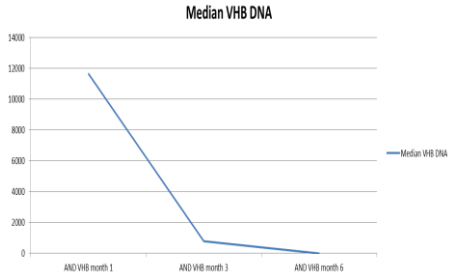

Figure 1. Cumulative rates of HVB DNA clearance of the sudy group during the first six months of therapy.

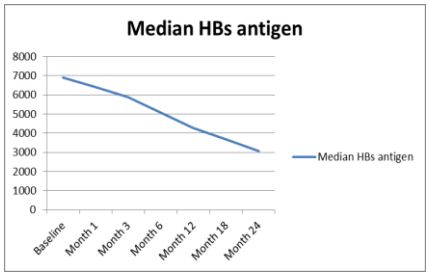

Figure 2 Median decreases of HBs antigen titers

CONCLUSIONS

The results from the patients in our study show that the decrease in HBs Antigen titers is very slow in most patients under nucleoside analogues treatment. On the other hand the HBV DNA decreased relatively fast in almost all patients. This suggests that nucleoside analogues offer a very low rate of seroconversion in HBs system and in a very long time but they are efficient in suppressing viral load and preventing complications.

The patients that cleared HBs antigen and the one who developed antibodies were HBe positive patients with very low baseline HBs antigen titers. In the patient that achieved HBs seroconversion, this was preceded by HBe seroconversion.

In the HBe negative patients the decrease in HBs antigen was slight and there were also patients that had an increase in HBs antigen titers while under treatment with nucleoside analogues, but just as the HBe positive patients they also cleared HBV DNA in a relatively short time. Correlations between HBs Antigen decrease and HVB DNA decreased were found only in HBe positive patients.

One patient did not clear HBV DNA and had increasing levels of HBs antigen. This turned out to be a case of resistance to Entecavir in a patient previously treated with Lamivudine for about three years before initiating Entecavir treatment.

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