



Research Article

A Study on Seroconversion in HBeAg Antigen Positive Chronic Hepatitis B Patients with Tenofovir Therapy

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Abstract

Introduction: Chronic Hepatitis B remains most important risk factor for developing cirrhosis, hepatic decompensation and hepatocellular carcinoma. So treatment with tenofovir of HbeAg positive Chronic Hepatitis B and seroconversion is the major concern.

Aim: To see the HbeAg and HbsAg loss after receiving tenofovic therapy for 6 and 12 months.

Methodology: A hospital based prospective study conducted on 50 chronic hepatitis B infection with HbeAg antigen positive patients between age group 12 to 60 years, receiving tenofovir (300mg) was taken for study. HbeAg, HbsAg and liver enzyme status was checked at the end of 6 months and 12 months.

Results: ALT level became normal (<19mg/ml) in 37.5 % of female patients at the end of 12 months study and in male it was 67.65%. Overall biochemical response was 58%. All patients were HbeAg reactive at the end of 6 months of tenofovir therapy but 48 percent became non reactive at the end of 12 months. Overall seroconversion was 36 percent at the end of 12 months.

Conclusion: There is statistical significant number of HbeAg loss, normalization of ALT level and HbeAg seroconversion at the end of 1 year. HBV DNA level became undetectable level in all sero converted groups.

Keywords: Chronic hepatitis B; Tenofovir; Seroconversion.

Introduction

Hepatitis B nowadays is the leading cause of chronic hepatitis worldwide especially in developing countries. In the Global Burden of Disease Study 2010, Hepatitis B Virus (HBV) was estimated to have resulted in 7.86 lakhs deaths, the vast majority being attributable to liver

cancer and cirrhosis.¹ Transmission of infection from a HBV carrier mother to her neonate accounts for the majority of new infections. Other less common sources are household contact with a HBV carrier, haemodialysis, exposure to infected healthcare workers, tattooing, artificial insemination and receipt of blood products or

organs. The number of HbsAg carrier in India has been estimated to be 40 millions. Overall HbsAg prevalences is 3 to 4 % among Indians.² Chronic Hepatitis B remains most important risk factor for developing cirrhosis, hepatic decompensation & hepatocellular carcinoma in developing countries. So it is important to diagnose and treat chronic Hepatitis B patients to prevent complications. Based on the presence of Hbe (envelope) antigen in serum of chronic hepatitis B (CHB) patients are classified into HbeAg positive & HbeAg negative sub groups. Currently treatments of chronic hepatitis B infection include interferon & antiviral drugs like nucleoside analogue and nucleotide analogue. Tenofovir disoproxil (nucleotide) an antiretroviral medication used to prevent and treat HIV is now extensively used to treat chronic hepatitis B.

Effective antiviral therapy for CHB would achieve control of viral replication, ALT (Alanine aminotransferase) normalization, HbeAg loss, seroconversion and a small number of patients may even achieve HbsAg clearance.

Response to tenofovir is measured by HbsAg titer decline, quantitative HBV DNA assay, change of LFT (liver function test) pattern, HbeAg antigen sero negativity. In our study we measured the success of tenofovir therapy by observing the HbeAg loss after predetermined period of 6 and 12 months. During the follow-up period we will look for HbeAg loss, LFT & HBV DNA level assay.

Methodology

The present study was a hospital base prospective study conducted at Medical Collage & Hospital, Kolkata from February 2015 to November 2016. Total 50 patients, who have active chronic hepatitis B infection and HbeAg seropositivity documented by clinical, biochemical were selected from patients attending Medicine outpatient department and liver clinic. Patients with decompensated chronic liver disease, CLD due to other causes like Hep C, alcohol and auto immune , patients with severe chronic co-morbid

illness , patients on any drug that can alter the test drug , patients with CHB infection who has other co infection (HIV) are excluded from the study. Chronic Hepatitis B patients are given treatment with drug Tenofovir only if HBV level > 20000 iu/ml, ALT above 2 times normal (Male 30 iu/L, Female 19 iu/L). After meeting the inclusion criteria and consent being taken from patients are put on Tab Tenofovir 300 mg daily after meal. All patients followed as monthly basis with clinical examination and routine lab test. The biochemical response assessed by liver enzymes, Virological response by HBV DNA (by PCR assay) and serological response by HbeAg status and appearance of anti HbeAb in patients with negative HbeAg at 6 months and 12 months interval. The appearance of anti Hbs antibody was the ultimate goal for therapy which was also checked by detecting HbsAb in Serum in HbsAg lost patients at the end of 6 and 12 months.

Statistical Analysis

Comparison between the parameters over time for overall cases and separately for cases who lost HBeAg after 12 months with those who did not, and within the first group, between those who were seroconverted and who were not is done using paired t test. Continuous variables like AST, ALT, ALP and HBV DNA (log 10 values) at different time points are expressed as Mean \pm Standard Deviation. Association between HBeAg (at initiation) and Anti Hbe(after study period) is captured using Pearson's Chi Square test for Independence of Attributes. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has significant statistical correlation.

Result

We carried out our study with a sample size of 50 persons, of which 34 (68 %) were male and 16 (32%) were female. All were aged between 12-60 years.

The pre-determined age limit of our study population was 12- 60 years. Maximum (19 out of 50; i.e. 38%) belong to 31-40 years age group, and the second highest group was of 21-40 years (14 people; 28%). Thus in our study, the majority were young adults.

This study population was started with 300 mg oral Tenofovir daily and was followed up at 6 month and 12 month, and the parameters studied were serum ALT level, AST level, HBV DNA (log 10 copies) to see their trend and also were looked for the loss of HBeAg or appearance of anti-HBe Ab.

Enzyme response was seen by ALT and AST, measured at 6 months and 12 months. Serum ALT level is used as a surrogate marker of disease activity and monitoring of treatment response in a patient of chronic hepatitis B. Normalization of serum ALT, (i.e. below 30IU/ml in male & below 19 IU/ml in female) is called biochemical response.

At the initiation, out of 16 females 15 had more than normal level of ALT (> 19 IU/ml) After end of study at 12 months of therapy 6 female patients (37.5%) out of 16 has achieved true biochemical response (ALT levels became less than 19 IU/ml).

Among the 34 male patients, all had more than normal ALT level (> 30 IU/ml) initially. At the end of 12 months 23 (67.65 %) has achieved biochemical response (ALT levels come down below or equal to 30 IU/ml).

So the overall biochemical response was seen in 58 % (29 out of 50) of patients after 12 month, at the end of our study.

The overall ALT response is significant from baseline to 12 months. The baseline mean was 119.24. After the initial 6 months the mean came down to 47.74 (p value 0.003) and after 12 months mean ALT value was 30.72 (p value < 0.001). This result denotes significant decline of ALT level over the study period (the p value at 12 months being < 0.001).

For the other liver enzyme AST, the population mean was 69.46 at 0 months, which came down

at the end of 6 months to 41.96 (p value 0.058, not significant) but in the next 6 months its decline was also significant, the mean being 35.04 at the end of 12 months (p value < 0.001).

Out of total 50 HBeAg reactive CHB patient of our study, at 6 months follow up no patients lost their HBeAg and at the end of 12 months of therapy, 24 patient (48%) became HBeAg negative (P value < 0.0001), on statistical analysis which is significant.

Of these 24 patients; 15 are male and 9 are female. Thus of 34 males, 15 (about 44%) underwent HBeAg nonreactive whereas of 16 females 9 (i.e. 56% approx...) was seen to have lost HBeAg from serum. So, the rate is found to be more in female.

Age wise if we see, maximum of the group who lost HBeAg comes under the age group 31-40 (38%)

Of the 24 patients who experienced HBeAg loss after 12 months, 18 was also found reactive for anti-HBe antibody thus having experienced seroconversion. Among 15 males, 11 had seroconversion and among 9 females 7 had seroconversion.

Thus, of 34 HBeAg positive males, 11 (about 32%) had seroconversion. In the female group, 7 out of 16 (44%) females experienced seroconversion.

Age wise, majority (44%) of the seroconverted individuals belong to the age group 31-40 years, next being the age group 21-30 years (33%).

As the patients were followed up for HBeAg seroconversion and 18 of those 24 patients (36%) showed HBeAg seroconversion by appearance of the anti HBe antibody in serum, the P value against which is < 0.0001, which is significant.

Thus overall 18 of the total 50 individuals; i.e. 36% (p value < 0.0001) showed HBeAg seroconversion by appearance of the anti HBe antibody in serum after 12 months of Tenofovir therapy, which is significant.

Virological response is monitored by the declining of HBV DNA over the time. Here we calculate the value of HBV DNA in log₁₀ base value. Normally virological response is defined by reduction of 4

log₁₀ of HBV DNA in HBeAg positive with time, in the patient receiving antiviral therapy. Here at the initiation, the mean HBV DNA log 10 copies was 7.12 ; after 6 months it came down to 3.49 log copies and after 12 months, it diminished to 0.6 log copies , where the p value of reduction of HBV DNA with time = <0.001.

There is also significant reduction of HBV DNA in both seroconverted and non- seroconverted group (p value is <0.001 in SC & non SC). The reduction in HBV DNA was more than 4 log 10 copies from the start of therapy to the end of our study period, which points towards the complete virologic response achieved by 1 year therapy with Tenofovir.

In the group that became non reactive for HBeAg at 1 year, mean value of DNA (log₁₀ copies) decline is 6.26, whereas the mean value in group which remained positive for HBeAg was 6.76 (p value <0.133), thus showing no significant difference.

Discussion

Numerous drugs are available to treat chronic Hepatitis B infection but no one has been proven to be effective sustained serological and virological response till now. As tenofovir is one of the latest drugs which in some studies has been shown to possess better efficacy in comparison with other oral and injectable drugs , we choose to carry on this study which also would speak about the efficacy and potency of tenofovir.

We selected 50 patients, who all fulfilled our inclusion criteria , and after proper demonstration and counseling and after having written consent from each of them, we put them on tenofovir 300 mg daily orally and subsequently follow-up done at liver clinic, Medical college, Kolkata. As per our aim in the study, we did investigate for HBsAg reactivity, serum HBe Antigen positivity, HBV DNA level and at baseline, 6 month and 12 month, anti HBe Antibody at 6 months and 12 months. We also monitor the liver function test (Liver enzymes). Our objectives were to look for rate of loss of HBeAg and rate of seroconversion

by appearance of anti- HBe Ab , to look for any loss of HBsAg. Besides we also studied the trend of liver enzymes and HBV DNA as the surrogate markers of drug response.

In our study among the 50 patients there were 16 females and 34 males, aged between 20 to 40 years. After 12 months of follow up, we analyzed the data obtained. It is seen that after 6 months there was no incidence of seroconversion for HBeAg and patients remained HBsAg positive whereas after 12 months in total, 24 patients (15 male and 9 female) have been seen to have lost HBeAg from serum, and this number is 48 % of the total population. 18 of these 24 patients also became anti HBe Ab reactive, i.e. experience seroconversion. Thus 36% of the total population underwent HBeAg seroconversion.

Our result has been compared with different study results, carried on CHB patients on tenofovir, with the aim to collect data on efficacy of the drug, mostly done as comparative studies along with any other oral or injectable anti viral(s). As previously mentioned, no direct study was done focusing on the rate of seroconversion. All the data on and rate of HBeAg or HBsAg loss and seroconversion rate have come as one of the supportive evidences of the efficacy of tenofovir , many a times superior to other drugs, which have been compared with tenofovir, in these comparative studies. Other parameters checked in these studies were response of HBV DNA and ALT on therapy.

In a double-blind study, carried out by Henry L.Y. Chan et al; nucleos (t) ide-naïve patients with high levels of hepatitis B virus DNA , positive for HBeAg and normal levels of alanine aminotransferase were randomly assigned to groups given either oral tenofovir disoproxil fumarate (TDF, 300 mg) and placebo (n = 64) or a combination of TDF (300 mg) and emtricitabine (200 mg, n = 62) for 192 weeks. The primary end point was proportion of patients with serum levels of HBV DNA <69 IU/mL at week 192. At the end of 192 weeks, HBeAg seroconversion occurred in

3 patients (5%), all in the TDF+placebo group; no patient had loss of hepatitis B surface antigen.³

To assess the efficacy of tenofovir in lamivudine resistant patients with a suboptimal response to LAM plus adefovir, a study carried out in Hong-Kong by Yang DH, 59 patients were randomized to switch to TDF (n = 28) or continuation with LAM plus ADV (n = 31). At week 48, in the tenofovir group, the HBeAg loss was 4%.⁴

In a comparative study on efficacy and safety of Tenofovir and Entecavir in Chronic Hepatitis B Virus infection by Weixia Ke, Li Liu et al HBeAg seroconversion rates 24 weeks post treatment was found to be a concern.⁵ The results of the two studies indicated that the pooled HBeAg seroconversion rate for the TDF group was 28%.

Batirel A et al studied on comparison of efficacy of tenofovir and entecavir, in which it was found that HBeAg seroconversion was 24% in the HBeAg reactive group, after a mean duration of 30 months. Only one patient in each group had hepatitis B surface antigen (HBsAg) clearance.⁶

A study carried out by Healthcoat EJ, Marcellin P et al. named "Three years efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B", showed better long-term efficacy and safety of TDF monotherapy in patients with chronic hepatitis B who were positive or negative for hepatitis B e antigen (HBeAg(+)) or HBeAg(-) and at week 144, 34% of the HBeAg reactive patients had been found lost HBeAg. Significantly more HBeAg-positive patients treated with tenofovir DF than those treated with adefovir dipivoxil had experienced loss of hepatitis B surface antigen (3% vs. 0%, P=0.02).⁷

Thus it was found, that the international data on HBeAg reactive patients show different rate of seroconversion in different studies, difference may be attributed to the viral factors such as genotype prevalent in a particular geographic area, the genetic build up of the subjects, and on other possible factors. Moreover, not all studies are carried on solely treatment naïve patients, rather

in many studies, patients, previously treated with another drug but unresponsive or resistant, were also included in the study populations. Lastly, study durations are variable, with some study results after a long follow up. Despite of these facts, it can be stated that our study result is comparable with these international data, including those which were carried on for almost the same duration as ours.

Measurement of serum HBV DNA load remains as an important tool for monitoring antiviral treatment outcomes for CHB patients who do not always experience seroconversion, although they have undergone with potent suppression of HBV replication, followed by treatment with regular antiviral treatment. In the current study, our results show among the 50 patients the mean HBV DNA level (log₁₀ copies/ml) came down from 7.12 at initiation to 0.6 at the end of study period, and this decline is well more than 4 log₁₀ copies that signifies complete virologic response. However no significant change is observed in the seroconverted group, and the decline in seroconverted and non-converted group is almost comparable.

Regarding biochemical response also we have seen that significant ALT decline occurred after the total period in all total population but without any significant difference between seroconverted and non-converted group.

In the reference studies also, biochemical and virologic response occurred in all patients, whether they lost their HBeAg or not and no gross difference is found in the serologic responder group. As for example in the comparative study by Hong Shi, Mingxing Huang, Guoli Lin, Xiangyong Li et al on efficacy comparison of Tenofovir and Entecavir in HBeAg-Positive Chronic Hepatitis B patients with high HBVDNA, it was found that at 48 weeks, the ALT and HBVDNA response is more in tenofovir group than entecavir group and HBV DNA suppression to undetectable levels by 48 weeks occurred 96.7% versus 86.4% respectively.⁸

In the study where tenofovir was compared with adefovir for chronic hepatitis B, carried out by Marcellin P, Heathcote EJ, et al, at 48 weeks, viral suppression occurred in 76 % of HBeAg positive patients and ALT normalization occurred in 68%, and it was significantly more than that compared to the adefovir group.⁷

In the study on comparable efficacy of tenofovir versus entecavir and predictors of response in treatment-naïve patients with chronic hepatitis B, Batirel A, Guclu E, Arslan F et al showed that the average period of ALT normalization and virologic clearance was around 20 months.⁹

In the comparative study which proved that tenofovir disoproxil fumarate is superior to lamivudine plus adefovir in lamivudine-resistant chronic hepatitis B patients; Yang DH, Xie YJ et al showed that at 48 weeks, CVR was 96% and ALT normalization was 89%.¹⁰

Thus it was seen from review of all the studies abroad and comparison with our study that results are almost comparable despite of variability in sample size, study duration and also of patient profile.

Conclusion

Thus in the final conclusion it can be stated that tenofovir as an monotherapy in treatment naïve HBeAg positive individuals, is a well efficacious drug resulting in acceptable level of HBeAg loss and subsequent seroconversion at 12 months, as well ensuring good biochemical and virologic response, without any incidence of serological breakthrough, and with good tolerance and economic acceptability. However further studies with larger sample sizes and prolonged follow up are needed for coming to a final conclusion

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