Original Article

A Study on Response of Tenofovir in Chronic Hepatitis B in Eastern India

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Background : Hepatitis B Virus (HBV) infection is a major public health problem worldwide as approximately 350 million have chronic HBV infection of which 15 to 40% may progress to Chronic Liver Disease and may further develop Hepatocellular Carcinoma.

In the registration trial Tenofovir, an oral nucleotide analog, polymerase inhibitor was found to be highly effective and potent with a sustained virological response. However in eastern India there are no landmark studies on Tenofovir.

Aims and Objectives : To evaluate the effect of Tenofovir as first line monotherapy on viral suppression and hepatic function in chronic hepatitis B patients with Chronic Liver Disease.

Materials and Methods : After fulfilling the inclusion criteria, 72 patients with chronic Hepatits B infection, were prescribed Tab Tenofovir (300 mg /day) for 1 year in this hospital based prospective study. Periodic follow-up with clinical, biochemical and virological assessment was done at 6 months and 1 year.

Results : Our study shows loss of HBsAg in 10 (13.88%) patients and HBeAg seroconversion was 81.81%. Biochemical and Child Pugh score improvement was statistically significant. At end of study total 42 (58.33%) achieved HBV DNA below detectable level (<3.8 iu/ml).

Conclusion : There was statistically significant improvement of clinical, biochemical,serological and virological parameters with minimum side effect and well tolerability after 1 year of therapy with Tenofovir in Chronic Liver Disease patients with chronic Hepatitis B infection. HBeAg seroconversion was high and sustained and the achievement of undetectable HBV-DNA was significant and almost similar in both HBeAg reactive & non reactive groups.

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Key words **: Chronic Liver Disease, Chronic Hepatitis B, Tenofovir, Hepatitis B Virus.**

Hepatitis B Virus (HBV) infection is a major public health problem World wide. Despite the availability of a highly effective vaccine there are 2 billion cases of HBV infection of which approximately 350 million have chronic HBV infection. The number of HBsAg carrier in India has been estimated to be over 40 million. Chronic HBV remains inactive in 60 to 70% cases; 15 to 40% infection may progress to Chronic Liver Disease (CLD) (cirrhosis) and may also progress to Hepatocellular Carcinoma (HCC).

Tenofovir, an oral nucleotide analog, polymerase inhibitor, appears to be the most potent of the HBV antivirals specially in HBeAg negative patients .In the registration trial tenofovir was found to be highly effective and potent in Treating Hepatitis B with sustained virological response and no resistance was noted in 4 years. In eastern India there are no landmark studies on Tenofovir, thus the purpose of this study is to evaluate the response of Tenofovir in chronic HBV infection and to find out biochemical, virological, serological and histological response of Tenofovir in chronic HBV infection at 6 months and at 1 year.

Editor's Comment :

- Hepatitis B infection is a major public health problem with approximately 350 million worldwide cases of chronic HBV infection.
- Our study found statistically significant improvement in clinical, biochemical, serological, and virological parameters with minimum side effects after 1 year of therapy with Tenofovir in chronic hepatitis B patients.

Materials and Methods

(I) **Study Area :** Medical College Hospital, Kolkata

(II) **Study Population :** Chronic Liver Disease patients due to HBV infection attending MOPD or admitted in MCH, Kolkata.

(III) **Study Period :** 1year

(IV) **Sample Size :** (n) 72.

(V) **Sample Design :**

All chronic liver disease patients (Fulfilling the inclusion & exclusion criteria) due to HBV infection attending Medicine Outpatients Department (OPD), admitted in wards who need treatment (according to ASSLD guideline) and gives written informed consent will be made part of this study.

(VI) **Inclusion Criteria :**

(1) Patients of Chronic Liver Disease (documented

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by clinical, biochemical and histological criteria) with HBV infection (detected by HBeAg, HBV DNA) who are treatment naive

(2) Age group of 12 to 60

(VII) **Exclusion Criteria :**

(1) Patients of Chronic Liver Disease due to other causes (Hepatitis C, Alcohol, Autoimmune Hepatitis)

(2) Patients with severe co-morbid illneses.

(3) Patients on any drug that can alter the test drug

(4) Patients with decompensated cirrhosis

(VIII) **Study Design :** Hospital based prospective study with 72 patients (n=72). In HBeAg positive patients treatment is given only if HBV DNA >20000 IU/ ml and AST, ALT above 2 times normal.

In HBeAg negative patients treatment is given only if HBV DNA >2000 IU/ ml and AST, ALT above 2 times normal (AASLD Guideline).

After inclusion criteria is met and written consent is obtained, patients were given tablet Tenofovir 300 mg daily after meal. All patients were followed on monthly basis at OPD with clinical examinations & routine laboratory tests.

The biochemical response was assessed on the basis of LFT with emphasis on total Bilirubin and liver enzymes. Biochemical breakthrough was considered as increase in ALT above 1.5 times upper limit of normal.

The virological response was assessed at baseline, at 6 month & at 12 month. Virological response was considered as undetectable HBV-DNA by PCR (<3.8 copies/ml), HBeAg seroconversion was considered as loss of HBeAg and appearance of antibody. Disappearance of HBsAg was the ultimate goal of treatment. Virological breakthrough was considered as the reappearance of detectable HBV-DNA after an episode of undetectable level or increase of HBV DNA one log from nadir.

(IX) **Parameters To Be Studied :**

(1) Clinical parameters

(2) Biochemical parameters

- (a) Liver Function Test
- (b) PT(in sec), INR
- (c) Complete haemogram
- (d) Sugar, Urea, Creatinine
- (e) ANA (when required)
- (f) Serum ceruloplasmin (when required)

(3) Microbiological parameters

- (a) HBsAg
- (b) HBeAg
- (c) HBV - DNA
- (d) HCV, HIV

(4) Radiological parameter

USG whole abdomen (liver echotexture, portal vein diameter, ascites, splenomegaly)

(5) Upper Gastrointestinal Endoscopy

(6) Histology where feasible

(X) **Study Tools :**

(A) **Clinical examination**

(i) Symptoms : Anorexia, Nausea, Vomiting, Hematemesis & Melena, Fatigue, Itching, Fever, Bleeding Tendencies, Jaundice, Swelling of Legs, Pain Abdomen, Distension of Abdomen, Skin Rashes, Joint Pain, Impotence.

(ii) Signs : altered consciousness, Hair Loss, Icterus, Pallor, Fetor Hepaticus, Palmar Erythema, Asterxis, JVP, Spider Angioma, Clubbing, Muscle Weakness, Pedal Edema, Petichae, Palpable Purpura, Ascites, Right Hypochondrial Tenderness, Hepatomegaly, Splenomegaly, Signs of Portal Hypertension.

(B) Biochemical Tests :

(i) Serum total bilirubin (Jendrassik & Grof method)

(ii) ALT [SGPT] (UV Kinetic IFCC Method)

(iii) AST [SGOT] (UV Kinetic IFCC method)

(iv) Serum alkaline phosphatase (PNPP method)

(v) Serum total protein (Biuret method)

(vi) Serum Albumin (BCG Method)

(vii) HBs Ag estimation by ELISA :

(viii) HBeAg test: double antibody sandwich immunoassay.

(ix) Anti –HCV antibody by ELISA

(C) Pathological Examination :

Liver biopsy and histological examination :

Liver biopsy is the traditional gold standard for evaluation of Chronic Liver Diseases. A complete physical examination & history, review of medications, and measurement of clotting parameters are essential.

(D) Virological examination :

HBV DNA assay : by Polymerase Chain Reaction (PCR) amplification

(E) Child Pugh Score :

Chronic Liver Disease is classified into Child-Pugh class A to C.

(XI) **Study Techniques :**

Tests to diagnose Chronic Liver Disease : Clinical examination, LFT, USG whole abdomen (W/A), PT/INR, Upper GI Endoscopy.

Tests to assess HBV activity: HBsAg, HBeAg, HBV DNA quantitative assay.

Tests to exclude other systemic diseases: Complete Hemogram, Blood Sugar, Urea, Creatinine, Chest X-ray, ECG, ANA in some cases.

Tests to exclude HCV & HIV : Anti HCV antibody, HIV ELISA

(XII) **Statistical analysis :** paired t test was used and the P-value <0.05 taken as significant.

(XIII) **Software used :** Graph pad prisom version 6 was used for statistical analysis. Microsoft Office Excel 2007 was used for tabulation, calculation and table & chart preparation.

Analysis and Results

We evaluated,100 patients of which 72 patients

fulfilled the inclusion criteria.

Finally 72 (56 male and 16 female) treatment naïve patients of chronic Hepatitis B were included in our study.

Patients who fulfilled the treatment requirement criteria were prescribed tab Tenofovir 300 mg /day O.D dose irrespective of HBeAg status (Table 1).

Table 1 — *Baseline Parameters*

Parameter	Average (n=72)
Bilirubin(mg/dl)	1.545
ALT (IU/L)	205.55
AST (IU/L)	193.58
ALP (IU/L)	266.80
Albumin (g/dl)	3.478
Globulin (g/dl)	3.347
CP SCORE	6.056

Response of Bilirubin :

At baseline average bilirubin was elevated. Decrease of mean total bilirubin was statistically significant both 6 months and 12 months (p value <0.05)(Table 2, Fig 1).

Table 2 — *Change of Total Bilirubin*

Time in month	Mean ± SEM	P value
Baseline	1.545 ± 0.1642 (N=72)	
6 months	0.9389 ± 0.05275 (N=72)	<0.0008
12 months	0.7750 ± 0.04205 (N=72)	<0.0001

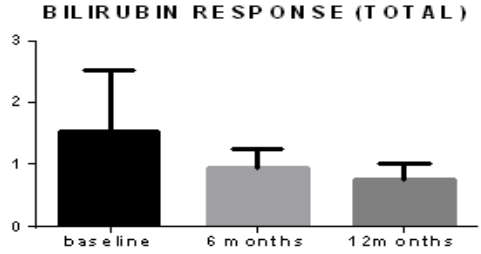

Fig 1 — Comparision of bilirubin at baseline, 6 months and 12 months

Response of ALT and AST :

At baseline every patients had ALT above normal range (male 30IU/L and female 19IU/L). At the end of 6 months 6(8.3%) patients ALT became normal and at end of study 46(63.88%) patients ALT became normal. P-value was significant both 6 months and 12 months.

At baseline AST of all patient was above normal (male 30IU/L and female 19IU/L) at 6 months 6 (8.3%) patients achieved normal AST and 12 months 50(69.44%) patients achieved normal AST level. Result was significant (Table 3, Figs 2&3).

Response of Child Pugh Score

CP score of all patients were calculated at baseline, 6 month and 12 months. Improvement of CP score was statistically **significant** as p value is <0.005 both 6 months and 12 months (Table 4).

Serological Response

At baseline 44 (61.11%)patients were HBeAg positive and 28(38.88%) were HBeAg negative.

Table 3 — *Change of ALT and AST In Study Population*

Time in months	Mean ± SEM	P value
Baseline of ALT	205.6 ± 23.43 (N=72)	
6months ALT	63.50 ± 4.565 (N=72)	< 0.0001
12 months ALT	30.57 ± 2.025 (N=72)	< 0.0001
Baseline of AST	193.6 ± 20.31 (N=72)	
6 months AST	60.39 ± 5.802 (N=72)	< 0.0001
12 months AST	30.23 ± 2.181 (N=72)	< 0.0001

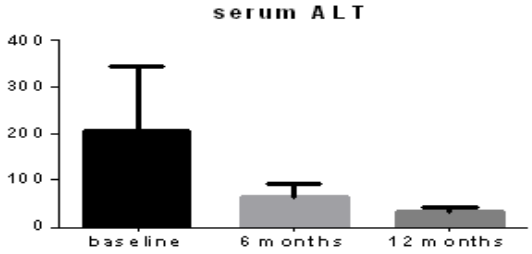

Fig 2 — comparison of ALT at baseline, 6 months and 12 months

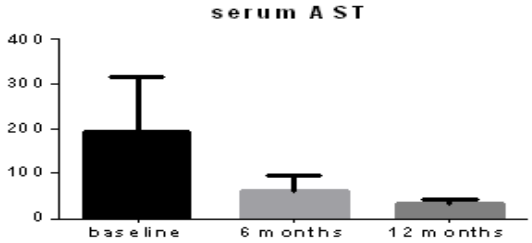

Fig 3 — comparison of AST at baseline, 6 months and 12 months

Table 4 — *Distribution of patients according to Child Pugh score (n=72)*

Time	CP class A	CP class B
Baseline	52(72.22%)	20(27.77%)
6 months	70(97.22%)	2(2.77%)
12 months	72(100%)	0(0%)

At 6 month 26 (59.09%) patients and at 12 months total 36 (81.81%) out of 44 HBeAg positive patients lost HBeAg status.

At end of study 10 (13.88%) patients lost HBsAg.

So there is high percentage of HBeAg and HBsAg loss during 12 months of therapy. No serological breakthrough has been reported.

Virological Response

HBV-DNA became below detectable level (<3.8 iu/ml) in 2 patiens (2.78%) at 6 month and 26(59.09%) patients in HBeAg positive (n=44) patients at 12 month.

16 patients (57.14%) achieved HBV DNA below detectable level (<3.8iu/ml) at 12 month in HBeAg negative patients (n=28). Overall HBV DNA below detectable level (<3.8iu/ml) at 12 month was in 42 patients (58.33%) which is highly significant.

Discussion

Cirrhosis develops as a result of hepatic inflammation and subsequent fibrosis in chronic

Hepatitis B infection. Patients with Hepatitis B virus (HBV) cirrhosis and high levels of serum HBV-DNA are more likely to develop liver failure and Hepatocellular Carcinoma (HCC). Spontaneous or drug-induced suppression of serum HBV-DNA is associated with biochemical and histological remission of liver disease. The mainstay of therapy for HBV cirrhosis is the inhibition of the replicative cycle of HBV in hepatocytes. In patients with HBV related cirrhosis, the issues in choosing a drug, such as efficacy, safety, incidence of resistance, method of administration and costs, are of particular concern. A major concern with long-term antiviral treatment are antiviral-resistant mutations. Emergence of antiviral-resistant mutations can lead to negation of the initial response, Hepatitis flares and hepatic decompensation. Use of the most potent agents as first-line remedy lowers the threat of resistance. Potency in suppressing HBV-DNA is the main factor in the choice of first-line therapy; Tenofovir constitute the most potent nucleoside analogues to date with the lowest rates of resistance. The aggregate efficacy and safety data now support the use of Tenofovir as a first line treatment option for nucleoside naive cases with compensated HBV cirrhosis.

There are very few studies of Tenofovir, especially in eastern India. In our study we included 72 chronic Hepatitis B infected, treatment naive patiens after fulfilling all inclusion criteria and written informed consent. They received 300 mg Tenofovir daily orally for 1 year. Periodical follow-up was done at 6 and 12 months.

Total bilirubin dropped significantly from baseline. At baseline bilirubin was elevated in 44 (61.11%) patients and at end of study only 18(25%) patient's bilirubin was above normal (bil ≤1 mg /dl)

ALT and AST decrease from baseline 6 months and 12 months were significant. ALT normalization at end of study was 59.09% in HBeAg positive patients and 64.28% in HBeAg negative patients.

There was significant improvement of CP score from baseline. P value was significant both 6 months and 12 months.

Among 44 HBeAg positive patients at baseline, 26 patients (59.09%) lost HBeAg at 6 month and 36 patients (81.81%) at 12 month.

HBsAg loss at end of study was in 10 patients (13.89%).

HBV DNA became below detectable level (<3.8iu/ml) in 2 patients (2.78%) at 6 month and 26(59.09%) patients in HBeAg positive (n=44) patients at 12 month.

16 patients (57.14%) achieved HBV DNA below detectable level (<3.8 iu/ml) at 12 month in HBeAg negative patients (n=28). Overall HBV DNA below detectable level (<3.8 iu/ml) at 12 month was in 42 patients (58.33%) which is highly significant .

No death occurred during the study period. No severe adverse effect was reported during one year follow up. Only 4(5.55%) patients had mild elevation of Serum Creatinine (<0.5 mg/dl)

Limitations : One of the limitations of this study is lack of histological evaluation as it was very difficult to motivate patients for liver biopsy. This type of study demands more time and number of patients. Resistance profile and mutation analysis are important evolving parameters but due to high cost, we could not perform it. More extensive epidemiological evaluation of patients could have been done.

Thus, further studies with a larger cohort of patients, especially comparison study with another antiviral drug or combination therapy is required.

Conclusion

There was statistically significant improvement of clinical, biochemical, serological and virological parameters with minimum side effects and well tolerability after 1 year of therapy with Tenofovir (300 mg /day) in Chronic Liver Disease patients with chronic hepatitis B infection.

HBeAg seroconversion was high and sustained, and the achievement of undetectable HBV DNA was significant and almost similar in both HBeAg reactive & non reactive groups.

We must say that further long term, multi-centric, multi-arm studies involving larger patient group is necessary in this field.

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