

ORIGINAL ARTICLE

Comparison of lamivudine - α -interferon combination and α -interferon alone treatments in adult patients with naive HBeAg negative hepatitis B

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ABSTRACT

Objectives: This study aimed to compare the efficacy of the α -interferon treatment with α -interferon plus lamivudine for cases of chronic hepatitis B.

Materials and methods: Sixty-one HBeAg negative naive chronic hepatitis B patients were randomly evaluated in two groups prospectively. In group 1, 30 patients were simultaneously given α -interferon 2a 9 MU, 3 days a week by s.c. injection plus lamivudine 100 mg a day for 12 months. In group II, there were 31 patients who only was received the same dosage of α -interferon and no lamivudine over the same period of time.

Results: In group 1 the initial mean value of alanine aminotransferase (ALT) was 144 ± 59 IU/L and decreased to 38.8 ± 19.3 IU/L; in group II, initial mean values of ALT was 141 ± 52 IU/L and decreased to 53.2 ± 14.7 IU/L at the end of 12th month of the therapy ($P < 0.05$). Hepatitis B virus DNA (HBV-DNA) clearance was obtained in 13 of 30 patients (43.3%) in group I patients and 15 of 31 (48.4%) in group II at the end of the therapy ($p = 0.692$). The number of patients with complete response was found to be 14 out of 30 (48.4%) in group 1 and 15 out of 31 cases (46.7%) in group II, six months after the end of the therapy ($P = 0.893$).

Conclusion: α -interferon and lamivudine combination therapy had a more beneficial effect than α -interferon monotherapy in normalization of ALT and clearance of HBV-DNA; however, the complete response rate at 6 months after the end of the therapy was not statistically significantly different between both groups. *J Microbiol Infect Dis 2011;1(2): 58-63*

Key words: Chronic hepatitis B, HBeAg negative, combination therapy, α -interferon, lamivudine

HBeAg negative erişkin hastalarda lamüvidin-alfa-interferon kombinasyonu ile tek başına alfa-interferon tedavilerinin karşılaştırılması

ÖZET

Amaç: Bu çalışmada kronik hepatit B hastalarında alfa-interferon ile alfa-interferon + lamivudin kombinasyon tedavisinin etkinliğinin karşılaştırılmasını amaçladık.

Gereç ve yöntem: Altmış bir HBeAg negatif naif, kronik hepatit B hastası, rastgele, ileriye dönük olarak iki gruba ayrıldı. Birinci grup, 30 hastaya aynı anda haftada 3 gün subkütan alfa-interferon 2a 9 MU ve günlük 100 mg lamivudin 12 ay boyunca verildi. 2. grup, 31 hasta ise sadece aynı dozlarda alfa-interferon aldı ve hiç biri lamivudin kullanmadı.

Bulgular: Oniki aylık tedavi sonunda, birinci grubun başlangıç ortalama alanin aminotransferaz (ALT) düzeyi 144 ± 59 IU/L'den, $38,8 \pm 19,3$ IU/L'e, ikinci grubun grubun başlangıç ortalama alanin aminotransferaz (ALT) düzeyi 141 ± 52 IU/L'den $53,2 \pm 14,7$ IU/L'e geriledi ($P < 0,05$). Tedavinin sonunda Hepatit B virüs DNA'sı (HBV-DNA) birinci gruptaki 30 hastanın 14'de (% 48,4) ve ikinci gruptaki 31 hastanın 15'de (% 46,7) negatifleşmiştir ($p = 0,692$). Tedavi sonrası altı aylık takiplerdeki kalıcı yanıt ise, birinci gruptaki 30 hastanın 13'de (% 43,3) ve ikinci gruptaki 31 hastanın 15'de (% 46,7) sağlandı ($P = 0,893$).

Sonuç: Alfa-interferon ve lamivudin kombinasyon tedavisi ALT normalleşmesi ve HBV DNA'nın temizlenmesinde alfa-interferon monoterapisinden daha yararlı etkisi olup, her iki grupta, tedavi sonrası altı ay sonundaki kalıcı yanıt oranları arasında istatistiksel olarak anlamlı bir fark saptanmadı.

Anahtar kelimeler: Kronik hepatit B, HBeAg negatif, kombinasyon tedavisi, alfa-interferon, lamivudin

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INTRODUCTION

More than 400 million people worldwide are chronically infected by the hepatitis B virus (HBV).¹ The number of the liver cancer cases estimated in a year is 530,000 in the world. On the other hand, 82% of these was caused by viral hepatitis infection which was associated with hepatitis B in 316,000 cases.² Chronic hepatitis B (CHB) infection is the primary cause of cirrhosis and hepatocellular carcinoma worldwide. Alfa-interferon is effective in patients with hepatitis B with long lasting response rate.³ Studies in Asia showed a much lower response rate at 10-20%.⁴⁻⁵ More than 50% of the patients with CHB infection do not respond to α -interferon treatment and require alternative therapies. Treatment with a different α -interferon preparation, administration of a different drug and combination therapies is a common treatment alternative.⁵

Lamivudine, an oral nucleoside analog, has been shown to cause suppression of hepatitis B virus DNA (HBV-DNA), a marker of active viral replication, in 93% of patients after 6 months of therapy when 100 mg doses is used.⁶ Quantitative hepatitis B surface antigen (HBsAg) concentrations also decrease during treatment. However, after withdrawal from this drug, an immediate rise in HBV-DNA to pretreatment levels was observed, followed by a more gradual increase in HBsAg concentrations.⁷ In a study from India, 45% percent of patients with CHB who achieve end-of-treatment response relapse and sustained viral response to lamivudine therapy is achieved in 14%.⁸ Recently, it has been claimed that α -interferon has a direct antiviral effect on chronic HBV infection, which may be additive to, or synergistic with lamivudine.⁹

There are two drugs approved for the treatment of chronic hepatitis B virus (HBV) disease: IFN- and lamivudine. Several randomized controlled trials have shown the efficacy of IFN- in the treatment of hepatitis B e antigen (HBeAg)-positive chronic hepatitis B.¹⁰⁻¹¹ These studies show that approximately 25-40% of the treated patients clear both the HBeAg and the HBV DNA from serum.¹⁰ In addition, lamivudine at a dosage of 100 mg daily for 52 weeks results in suppression of HBV DNA in the majority of patients, but relapse is common. Sustained suppression of HBV DNA and HBeAg occurs in only 16% of pa-

tients, and the degree of lamivudine resistance increases with the duration of therapy.^{12,13}

The HBeAg negative form of chronic hepatitis B (CHB) predominates in the Mediterranean area and has a rising frequency in Europe and North America. The HBeAg negative CHB is characterized by frequent exacerbation of hepatitis, which probably increases the risk of cirrhosis, hepatocellular failure, and hepatocellular carcinoma.¹⁴

The aim of the present study is to compare the efficacy of the treatment with α -interferon alone and the combination of α -interferon in lamivudine in HBeAg negative CHB infection at the end of the treatment and six months after therapy.

MATERIALS AND METHODS

This study was carried out at the Department of Clinical Microbiology and Infection Diseases, Dicle University Medical Faculty. The diagnosis of all patients was confirmed after a thorough laboratory investigation for their symptoms of icterus, abdominal pain, fatigue, loss of appetite or prior detection of elevated alanine aminotransferase (ALT) values and/or family history of HBV infection.

Inclusion criteria were the presence of the HBsAg in serum for at least 6 months, presence of hepatitis B early antibody (Anti-HBe), absence of hepatitis B surface antibody (anti-HBs), absence of hepatitis B early antigen (HBeAg), ALT values (the upper limits of the normal value is 40 IU/L) more than 1.5 times the normal upper limit, presence of HBV-DNA and histological evidence of chronic hepatitis disease on liver biopsy taken within 6 months of enrollment.

Patients were excluded at screening if they had been treated previously with IFN or had received antiviral or immunosuppressive medications; if they had tested positive for antibody to hepatitis C virus, hepatitis D virus, or HIV; if they had other causes of chronic liver disease; if they drank >40 g of alcohol per day; if they had evidence of hepatocellular carcinoma; if they had decompensated liver disease (serum bilirubin level, >2.5 times greater than the ULN; prothrombin time prolonged by >3 s; serum albumin level less than the lower limit of normal; or a history of ascites, variceal hemorrhage, or hepatic encephalopathy); or if they had any contraindications specified for use of IFN. Patients also were

excluded if they were pregnant; if they had a total leukocyte count of <2500 cells/mm³, a neutrophil granulocyte count of <1000 cells/mm³, a platelet count of $<100,000$ cells/mL, or a hemoglobin level of <10 g/dL; or if they were unable to provide informed consent.

To determine viral load prior to the therapy, HBV-DNA was detected before treatment by solution hybridization technique (Hybrid Capture, Digene Diagnostics, Beltsville, MD, USA; cut-off, 5 pg/mL). Hepatitis B virus DNA was monitored on the twelfth and eighteenth months following treatment by polymerase chain reaction method.

For histological diagnosis, liver needle biopsy was carried out with ultrasonography guidance. The biopsy material was kept in 10% formaldehyde solution and evaluated by a pathologist experienced with liver pathology. All specimens were graded according to Knodell histological activity index (HAI).¹⁵

Complete blood count, biochemical and hepatitis serological (HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-Delta) studies were re-evaluated once a month for the first 2 months and then every other month, end of the treatment and six months after the end of the treatment. Hepatitis serology was studied with Tecan Minilyzer enzyme-linked immunosorbent assays (ELISA) apparatus and third generation ELISA (EQUIPAR, Saronno, VA, Italy) reagent was used. During follow-up, the probable side-effects of α -interferon and lamivudine were evaluated and considered to determine whether to continue the therapy.

Sixty-one HBeAg negative naive chronic hepatitis B Patients were evaluated in two groups prospectively. The patients were randomized in consecutive order to one of the two treatment arms. In group 1, 30 patients were simultaneously given α -interferon 2a 9 MU, 3 days a week by subcutaneous injection plus lamivudine 100 mg a day for 12 months. In group II, there were 31 patients who received the same dosage of α -interferon and no lamivudine over the same period of time. Serological, virological and biochemical results were compared between both groups at the end of the treatment and 6 months after the end of therapy. Complete response at the end of the therapy was accepted as the clearance of HBV-DNA and the normalization of ALT.

Before applying this protocol, informed consent was taken and sufficient information was

given to the patient's parents about the disease and the treatment procedure. The local ethical committee approved our study.

Results were expressed as mean \pm SD. The Chi Square test was used to compare categorical samples. If the total sample size and the expected values were smaller than five, Fisher's exact test was used. Values of $P < 0.05$ were considered statistically significant. Statistical analysis was carried out by SSPS version 15.0 programs.

RESULTS

Sixty-one adults between 27 and 44 years of age were enrolled. Mean ages were 36.2 ± 5.9 years and 36.9 ± 6.7 years in groups 1 and 2, prospectively (Table 1). In group 1, the initial mean value of ALT was 144 ± 59 IU/L and decreased to 38.8 ± 19.3 IU/L at the end of twelfth month of therapy ($P < 0.05$) and at the end of the first year, normalization of ALT was obtained in 15 out of the 30 patients (50%). In a follow-up session at the end of a 6-month period after treatment, 14 patients had normal ALT values (46.7%). In group II, initial mean values of ALT was 141 ± 52 IU/L and decreased to 51.2 ± 14.7 IU/L at the end of twelfth month of the therapy ($P < 0.05$). Normalization of ALT was obtained in 14 out of the 31 patients (45.2%) in this latter group at the end of the first year. In a follow-up session at the end of a 6-month period after treatment with group II, ALT values increased again 1.5 upper limits of normal value in one patient. The final rate of ALT normalization after the 6 months was 42%. The difference in ALT normalization was statistically not significant at 6 months after the therapy in both group 1 and group II ($P > 0.05$).

Table 1. Characteristics of patients with chronic hepatitis B who were treated with α -interferon and lamivudine combined therapy or α -interferon monotherapy

Characteristics	Group I (n=30)	Group II (n=31)
Sex (% male)	63	84
Age (mean \pm SD)	36.2 ± 5.9	36.9 ± 6.7
Initial ALT (IU/L)	144 ± 59	141 ± 52
ALT > 100 (IU/L) (n)	17	19
HBV-DNA (mean \pm SD)	3015 (37-4058)	2982 (51-4218)
HAI (mean)	8.1	7.9

During a follow-up session 6 months after therapy were also. In both groups, normalization of ALT was achieved in patients. At the end of the treatment HBsAg clearance and anti-HBs seroconversion were not maintained.

The initial titer of HBV-DNA was higher than 500,000 copy/mL in 23 of the 30 patients in group 1 and in 25 of 31 patients in group II ($P > 0.05$). In group 1, HBV-DNA clearance was obtained in 13 of 30 patients at the end of the therapy, and only two patients relapsed with an increase of HBV-DNA greater than 500,000 copy/mL after 6 months of follow-up after therapy. In group II, HBV-DNA clearance occurred in 15 patients at the end of treatment. However, HBV-DNA titer was re-elevated in 12 patients at the 6 months after the end of therapy. The differences between the two groups were statistically significant in the means of HBV-DNA clearances both at the end of the therapy and 6 months after the therapy ($P=0.042$).

Before therapy, the mean of Knodell HAI of liver biopsies was 8.1 in group 1 and 7.9 in group II ($P > 0.05$). The number of patients with sustained virological responses was 14 out of 30 cases (46.7%) in group 1 and 15 out of 31 cases (48.4%) in group II. There was no statistically significant difference between both groups ($P > 0.05$). Patients tolerated the treatment well. Therapy was continued, although some patients displayed typical minor side-effects such as a flu-like syndrome and gastrointestinal symptoms. No patients developed severe neutropenia, thrombocytopenia or any complication of bone marrow suppression.

DISCUSSION

Chronic liver disease during HBV infection is major cause of death worldwide, linked mainly to complications of cirrhosis and hepatocellular carcinoma. The age of acquisition, sex, HBV-DNA level in serum, histologic activity, ethnic group, immune status, viral mutations and coexisting viral infections all influence the rate of development of cirrhosis in hepatitis B.¹⁶ Alfa-interferon and lamivudine were being used for treatment of chronic hepatitis B infection while this study was in progress. Beside the rapid scientific development; entecavir, tenofovir, telbuvudine and other new antiviral drugs have been used for treatment recently. Due to its antiviral and immunomodula-

tory properties, α -interferon is the most promising therapeutic agent for the treatment of various CHB infections. The currently recommended regimen for α -interferon is 9 MU given three times a week by s.c. injection for six up to 12 months. Response as described by clearance of viral DNA and normalization of ALT is observed in 30-45% of adult patients with CHB infection receiving α -interferon.¹⁷

The development of new nucleoside analogs, that inhibit the HBV reverse transcriptase activity, such as lamivudine, famciclovir and others, has provided recently an alternative to interferon therapy for CHB infection. Lamivudine is a deoxycytidine analog that is active against hepatitis B virus. In patients with CHB, lamivudine profoundly suppresses HBV replication. Clinically significant improvements in liver histology and biochemical parameters were obtained with lamivudine in randomized trials in HBeAg negative patients with CHB and compensated liver disease.¹⁸ The major advantages of lamivudine are good tolerability, oral route of administration and safety in patients with hepatic decompensation. The major disadvantage is drug resistance, which is observed with increasing frequency following prolonged administration.¹⁹

In the new millennium, combination therapy studies have accelerated in CHB infection. Many studies have focused on combination therapy with one or more nucleoside/nucleotide analog and an immune-modulating agent, such as interferon or a therapeutic vaccine. This combination may act synergistically against HBV and delay or prevent the development of drug-resistant mutants.²⁰ Up until now the best treatment modality has been unknown in patients with CHB infection.

Recent studies have proposed that α -interferon has a direct antiviral effect on chronic HBV infection, which may be additive to, or synergistic with lamivudine.⁹ In a study from Germany, there was resistance to lamivudine monotherapy in CHB patients with liver transplantation, but HBV-DNA clearance has occurred when interferon treatment was combined to lamivudine to obtain a synergistic effect.²¹

Until now, the short-term use of α -interferon alone or in combination with lamivudine has not been reported to be effective for treatment of chronic hepatitis B infection. Data on combination therapy are few and are restricted to studies

of courses of treatment of 4-6 months' duration. Moreover, many published trials of combination therapy provide little support for the use of the α -interferon and lamivudine combination. Also, the design of the initial studies of combination therapy may not have been optimal for showing the full effects of combination therapy.^{22,23} On the other hand, in many controlled trials, it has been shown that there was an increased clearance of HBV DNA after prolonged α -interferon therapy, compared with treatment for the standard period of 16 weeks.²⁴

Karabay et al. demonstrated that administration of combination therapy for 18 months to HBeAg-negative patients induces rapid inhibition of viral replication, normalization of liver function, and histological evidence of improvement in liver disease. They found a rate of sustained virological response (50% of patients) after 18 months of combination therapy and 38% of the α -interferon monotherapy ($p > 0.05$).²⁵ In our study we found the rate of sustained virological response 46.7% after 12 months of combination therapy and 48.4% of the α -interferon monotherapy. The rate of SVR was similar in the both of treatment groups.

In another study, α -interferon plus lamivudine treatments were compared with interferon was given alone. In this trial 51 cases with typical chronic hepatitis B infection were enrolled, 49% receiving interferon alone and 51% receiving the combination of two drugs showed beneficial response. Although, the response rate with combination therapy (21%) was statistically significant, the authors stressed that combination therapy may be not more effective than the monotherapy (12%).²⁶ In our study; the response rate in combination therapy was not higher than monotherapy ($P > 0.05$).

In the present study, the combination of α -interferon plus lamivudine were compared with α -interferon alone in adults with chronic hepatitis B infection; there were statistically significant differences between both groups in normalization of ALT, with a more beneficial effect in the group with combination therapy. However, the complete response rates were not statistically significantly different between both groups.

REFERENCES

1. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337:1733-1745.

2. WHO. World Health Report 1996: fighting disease, fostering development, Geneva: World Health Organization, 1996.
3. Yao F, Gish RG. Treatment of chronic hepatitis B: new antiviral therapies. *Curr Gastroenterol Rep* 1999; 1: 20-26.
4. Gurkan F, Kocak N, Ozen H, Yuca A. Comparison of standard and high dosage recombinant interferon alpha 2b for treatment of children with chronic hepatitis B infection. *Pediatr Infect Dis J* 2000; 19: 52-56.
5. Yalcin K, Degertekin H, Yildiz F, Celik Y. Comparison of 12-month courses of interferon-alpha-2b-lamivudine combination therapy and interferon-alpha-2b monotherapy among patients with untreated chronic hepatitis B. *Clin Infect Dis* 2003;36:1516-1522.
6. Nevens F, Main J, Honkoop P et al. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. *Gastroenterology* 1997;113:1258-1263.
7. Dikici B, Bosnak M, Bosnak V et al. Comparison of treatments of chronic hepatitis B in children with lamivudine and α -interferon combination and α -interferon alone. *Pediatrics Int* 2002; 44: 517-521.
8. Thakur V, Sarin SK, Rehman S et al. Role of HBV genotype in predicting response to lamivudine therapy in patients with chronic hepatitis B. *Indian J Gastroenterol* 2005;24:12-15.
9. Mutimer D, Dowling D, Cane P et al. Additive antiviral effects of lamivudine and α -interferon in chronic hepatitis B infection. *Antivir Ther* 2000; 5: 273-277.
10. Wong DKH, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen positive chronic hepatitis B. *Ann Intern Med* 1993; 119:312-323.
11. Hoofnagle JH, Peters M, Mullen KD, et al. Randomized, controlled trial of recombinant human-interferon in patients with chronic hepatitis B. *Gastroenterology* 1988; 95:1318-1325.
12. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; 341:1256-1263.
13. Lai CL, Chien RN, Leung NWY, et al. A one year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998; 339:61-68.
14. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2001; 34: 617-624.
15. Knodell RG, Ishak KG, Black WC et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431-435.
16. Ruiz Moreno M. Chronic hepatitis B in children: natural history and treatment. *J Hepatol* 1993; 17: 64-66.
17. Hoofnagle JH, Di Bisceglie AM. The treatment of chronic viral hepatitis. *N Engl J Med* 1997; 336: 347-356.
18. Shi M, Wang RS, Zhang H, Zhu YF, Han B, Zhang Y, Jin LJ, Yang ZJ, Xu YP. Sequential treatment with lamivudine and interferon-alpha monotherapies in hepatitis B e antigen-negative Chinese patients and its suppression of lamivudine-resistant mutations. *J Antimicrob Chemother* 2006; 58: 1031-1035.
19. Pessoa MG, Wright TL. Update on clinical trials in the treatment of hepatitis B. *J Gastroenterol Hepatol* 1999; 14: S6-11.
20. Regev A, Schiff ER. Drug therapy for hepatitis B. *Adv Intern Med* 2001; 46: 107-135.
21. Seehofer D, Rayes N, Berg T et al. Additional interferon alpha for lamivudine resistant hepatitis B infection after liver transplantation: a preliminary report. *Transplantation* 2000; 69: 1739-1742.

22. Terrault NA. Combined interferon and lamivudine therapy: is this the treatment of choice for patients with chronic hepatitis B virus infection? *Hepatology* 2000; 32:675-677.
23. Lok AS, Heathcote J, Hoofnagle J. Management of hepatitis B: 2000-summary of a workshop. *Gastroenterology* 2001; 120:1828-1853.
24. Janssen HLA, Gerken G, Carreno V, et al. Interferon alpha for chronic hepatitis B infection: increased efficacy of prolonged treatment. *Hepatology* 1999; 30:238-243.
25. Karabay O, Tamer A, Tahtaci M, Vardi S, Celebi H. Effectiveness of lamivudine and interferon-alpha combination therapy versus interferon-alpha monotherapy for the treatment of HBeAg-negative chronic hepatitis B patients: a randomized clinical trial. *J Microbiol Immunol Infect* 2005;38:262-266.
26. Economou M, Manolakopoulos S, Trikalinos TA, Filis S, Bethanis S, Tzourmakliotis D, Avgerinos A, Raptis S, Tsianos EV. Interferon-alpha plus lamivudine vs lamivudine reduces breakthroughs, but does not affect sustained response in HBeAg negative chronic hepatitis B. *World J Gastroenterol* 2005;11:5882-5887.