Lamivudine in chronic hepatitis B virus

Hisham O. Akbar, *MbChB, MD.*

ABSTRACT

Objective: To access the effect of one year treatment with Lamivudine, and its durability on patients with compensated chronic hepatitis B.

Methods: Thirty-six patients with hepatitis B surface antigen positive, hepatitis B virus deoxyribonucleic acid (DNA) positive, were treated with 100 mg Lamivudine daily for one year, irrespective of their liver enzymes level, alanine aminotransferase or hepatitis B envelope antigen status. Patients with normal alanine aminotransferase and negative hepatitis B virus DNA at the end of the treatment were responders and those with persistent response 16 weeks off treatment were considered having durable response.

Results: Three patients dropped out and 33 patients completed the study. Eight patients (24.2%) had normal alanine aminotransferase, 25 patients (75.8%) had increased alanine aminotransferase. Thirty patients (90.9%) were hepatitis B envelope antigen negative. Two patients (25%) with initial normal alanine aminotransferase and 7 patients (28%) with increased enzyme level, responded at end of treatment. One patient with positive hepatitis B envelope antigen responded to treatment. Only one patient relapsed during follow-up period off treatment.

Conclusion: Lamivudine is associated with durable response in patients with hepatitis B virus and negative hepatitis B envelope antigen. Longer treatment for more than one year may be required to improve response rates.

Keywords: Lamivudine, liver enzymes, hepatitis B virus.

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Hepatitis B virus (HBV) infection is a common universal problem. Over 350 million people worldwide are presently infected with HBV.[1] Clinical presentation and outcome of infection depends on the age at infection, degree of virus replication and host immune status. Chronicity occurs in 90% in patients with peri-natal transmission and in 5%-10% when HBV acquired during adulthood.[2,3] Patients chronically infected are at high risk for developing liver cirrhosis, liver failure and hepatocellular carcinoma.

Alpha interferon was the first drug approved for treatment of HBV to suppress viral replication with 30%-40% response rate in selected group of patient.[4] Patients with suboptimal response to interferon include those with peri-natal infection, patients with precore mutant strains or with normal transaminase levels. In the Kingdom of Saudi Arabia (KSA), hepatitis B surface antigen (HBsAg) carrier rate was estimated 8.3% in 1980s,[5,6] however, with improved socioeconomic status and new born immunization programs, the prevalence among vaccinated Saudi children dropped to 0.3% in 1998.[7] Prevalence among Saudi blood donors decreased to 4.4% in 1997 and 3.4% in 1999.[8] The most frequent mode of acquiring HBV in KSA is perinatally and the most frequent strain is hepatitis B envelope antigen-negative, (HBeAg) HBV DNA positive (presumed precore variants).[7]

From the Department of Medicine, King Abdul-Aziz University Hospital, Jeddah, *Kingdom of Saudi Arabia*.

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Address correspondence and reprint request to: Dr. Hisham O. Akbar, Assistant Professor/Consultant Gastroenterologist, Department of Medicine, King Abdul-Aziz University Hospital, PO Box 80215, Jeddah 21589, *Kingdom of Saudi Arabia*. Tel. +966 (2) 6408272/6408243. Fax. +966 (2) 6408315. E-mail: gastro20000@yahoo.com

Lamivudine, a nucleoside analogue active in suppressing HBV replication is less expensive and associated with fewer side effects as compared to interferon.[9] In this study lamivudine was used to access its effect in Saudi patients infected with HBV.

Methods. This is a prospective study where Saudi adult patients (18 years - 60 years) referred with HBsAg and HBV DNA positive; irrespective of their liver enzymes (aspartate aminotransferase (AST)/alanine aminotransferase (ALT)) level were included. Patients were never treated with interferon or lamivudine (naïve). Those with decompensated liver disease (jaundice, ascites, lower limb edema, previous history of hepatic encephalopathy or bleeding esophageal varices) or patients coinfected with hepatitis C virus (anti HCV positive), hepatitis D virus or coexisting other chronic liver disease were excluded from the study. On meeting the inclusion criteria and after explaining the possible side effects of lamivudine, patients included had a written consent. Patients were advised to have liver biopsy to access the degree of liver involvement. Liver biopsy results reviewed by one pathologist were reported as mild (Grade 2/Stage one), moderate (Grade 3/Stage 2), severe (Grade 4/Stage 3) and cirrhosis (Stage 4). Those who refused liver biopsy were also included in the study. Pre-treatment HBV DNA measurement using branched signal amplification assay, (Chiron diagnostics) was carried out and repeated at the end of treatment (12 months) and 4 months off treatment. Lamivudine 100 mg was given orally daily for 12 months. Monthly follow up with serial complete blood count, liver enzymes (ALT) and full clinical evaluation including development of new symptoms was carried out in the clinic until end of treatment. Four months off treatment liver enzymes were repeated together with HBsAg, HBV DNA and HBeAg tests in previously positive patients. Patients with normal ALT and negative HBV DNA at the end of treatment were considered as responders. Partial responders were those patients with negative HBV DNA with increase ALT at end of treatment.

Statistical package for social sciences (SPSS), was used for analysis. Descriptive statistics were carried out. P-value was set at <0.05 for statistical significance.

Results. Thirty-six Saudi patients with HBsAg and positive HBV DNA were followed at King Abdul-Aziz University Hospital, Jeddah, KSA from April 1998 through to March 2001. Three patients were not included in the study because they failed to follow-up (2, 4 and 5 months). Only 33 patients completed the study as designed. Patient's characteristics are summarized in **Table 1**. There were 26 male patients (78.8%) and 7 female patients (21.1%). Mean age was 36.2 years (18 years - 54 years). Eight patients (24.2%) had initial normal ALT (group A) and 25 patients (75.8%) had increase ALT (group B). In group B, 12 patients (48%) had <2 fold increase and 13 patients (52%) had >2 fold increase in liver enzymes. 30 patients (90.9%) were HBsAg positive, HBeAg negative, HBV DNA positive (presumed pre-core mutant) and 3 patients (9.1%) in group B were HBsAg positive, HBeAg positive, HBV DNA positive. Liver biopsy was carried out in 20 patients (60.6%) and the remaining 13 patients (39.4%) refused. Mild hepatitis was found in 4 patients (20%), 10 patients (50%) had moderate hepatitis, 4 patients (20%) had severe hepatitis and 2 patients (10%) had liver cirrhosis. Five patients in group A had liver biopsy; 3 had mild hepatitis and 2 patients had moderate hepatitis while 15 patients in group B had liver biopsy where one patient had mild hepatitis, 8 patients had moderate hepatitis, 4 patients had severe hepatitis and the other 2 patients had liver cirrhosis (**Table 2**).

Complete response as defined by clearance of HBV DNA from serum and normal liver enzymes at the end of treatment, was achieved in 9 patients out of 33 patients (27.3%); complete response rate was 25% (2 patients out of 8 patients) in group A and 28% (7 patients out of 25 patients) in group B (P>0.05). Response rate among group B patients with <2 fold increased ALT was 25% (3 patients out of 12 patients) and 30.8% (4 patients out of 13 patients) in those with >2 fold increase (P>0.05). Mean HBV DNA in responders was 100.7meq/ml and 283.3meq/

Table 1 - Patients' characteristics.

ALT/Sex	Male	Female	Total
Normal ALT	5	3	**8**
ALT < x2	10	2	**12**
ALT > x2	11	2	**13**
ALT - alanine aminotransferase			

Table 2 - Liver history in group A and B.

Histology	Group A	Group B	Total
Mild	3	1	**4**
Moderate	2	8	**10**
Severe	0	4	**4**
Cirrhosis	0	2	**2**
Refused	3	10	**13**

Table 3 - Patients response during and after lamivudine.

Response/ Group	HBV DNA neg (During treatment)	HBV DNA neg Normal ALT (During treatment)	HBV DNA neg Normal ALT (Post treatment)
Group A	2 patients (25)	2 patients (25)	2 patients (25)
Group B	9 patients (36)	7 patients (28)	6 patients (24)
Total	**11 patients (33.3)**	**9 patients (27.3)**	**8 patients (24.2)**

HBV - hepatitis B virus, DNA - deoxyribonucleic acid
ALT - alanine aminotransferase

Table 4 - Normalization of ALT in group B patients.

Patient	ALT level	ALT normalization (Months)	ALT level (4 months after treatment)
One	>x2	4	Normal
2	<x2	7	Normal
3	>x2	3	Normal
4	<x2	6	Increased
5	<x2	4	Increased
6	>x2	5	Normal
7	<x2	6	Normal
8	>x2	7	Normal
9	<x2	4	Increased
10	<x2	5	Increased

ALT - alanine aminotransferase

ml in non responders (P=0.007). Mean HBV DNA in group A was 246.4 meq/ml and 234.7meq/ml in group B (P>0.05).

Partial response as defined by clearance of HBV DNA from serum with persistently increase ALT at end of treatment was achieved in 2 patients in group B, while 3 patients in group B had normal ALT with positive HBV DNA during treatment and increased during follow-up off treatment (4 months). One patient in the same group after initial normalization of ALT had increased ALT less than pre-treatment level that occurred at 10 months on treatment. Only one patient with positive HBeAg responded to treatment with seroconversion (negative HBeAg, positive hepatitis B envelope antibody, (HBeAb)). Undetectable HBV DNA at the end of treatment was found in 11 patients (33.3%) and in 8 patients (24.2%) after stopping treatment **(Table 3)**. 40% (10 patients out of 25 patients) with pretreatment increased liver enzymes (group B) had normal ALT in response to lamivudine at the end of treatment while 6 patients only (24%) had normal ALT after stopping treatment (4 months). Alanine aminotransferase normalization occurred at 3 months - 7 months during treatment (**Table 4**). Only one responder in group B and all partial responders (2 patients) relapsed with positive HBV DNA after stopping lamivudine. Side effects suggestive of peripheral neuropathy during treatment developed in one patient in group B that responded to vitamin B complex and none of the other 32 patients had any significant new symptom.

Discussion. Lamivudine has been studied in a wide variety of patients with HBV[9,10] and since its approval it has increased the options for treatment of patients with HBV. Variables used to access its effect were histological improvement (decrease of 2 or more points in Knodell score), normalization of liver enzymes during treatment; decrease HBV DNA beyond detection level (using non polymerase chain reaction dependant assays) and HBeAg seroconversion. Fifty-two percent - 56% of patients developed clinically significant reduction in Knodell necroinflamatory score using 100 mg lamivudine for 52 weeks, liver enzymes normalized in 41%-72%, 30% had HBeAg loss and 17% developed HBeAg seroconversion which was durable in 80% of cases after 16 weeks follow-up, off treatment.[9,11] Histological improvement assessment in this study was not possible since repeat liver biopsy was not performed. In addition only 3 patients had positive HBeAg and one patient (30%) responded to treatment with seroconversion (HBeAb positive). However this number of patients is very small to compare to similar response results of similar group of patients. Ninety point nine percent of patients in this study were HBeAg negative, HBsAg positive, HBV DNA positive (presumed precore mutant strains) which is similar to most patients with HBV in the Mediterranean region (98%).[12] This group of patients usually does not respond to interferon since the majority relapses,once treatment is stopped which limits treatment options available for them to lamivudine and other experimental nucleosides analogues. Lamivudine effect on patients with precore mutant virus has been the subject of different studies, which showed that 63% of patients had decrease in HBV DNA below detection level, 60% had significant reduction in Knodell necroinflamatory score,[10,13] 11% had complete response, 30% had undetectable HBV DNA level and 17% had persistent normal liver enzymes 24 weeks after discontinuing lamivudine.[9,14]

Decrease in HBV DNA below detection level in this study in patients with pre core mutant virus was 33.3% (10 patients out of 30) at the end of treatment

and 23.3% (7 patients out of 30) after discontinuing lamivudine. This difference in the results with the international reported results probably is due to the different available methods for HBV DNA assays with poor inter assay standardization and intra-assay variations. A low HBV DNA level was the only variable in this study that statistically affected response to lamivudine. Persistent normal ALT in response to treatment in patients with pre core mutant virus and pre treatment increased ALT was 40.9% (9 patients out of 22) at the end of treatment and 22.7% (5 patients out of 22) 4 months after treatment. The difference in response between end of treatment and after treatment probably as of lamivudine suppress replication of the virus but did not reduce the level of intracellular HBV, which persists in the form of covalently closed circular DNA (cccDNA) leading to relapse after discontinuing lamivudine.[15] Degree of increased liver enzymes was reported to be the strongest predictor for patient's response.[9] Patients with more than 2 fold increased ALT in this study had a higher response (39.4%) compared to patients with less than 2 fold increased or normal ALT (36.4%, 24.2%), which, did not reach statistical significance. Several studies showed direct proportion between HBeAg seroconversion and pre treatment ALT level, where HBeAg seroconversion rates for patients with pre-treatment ALT levels within normal, one - 2 times, 5 times or more than 5 times normal were 2%, 9%, 21% and 47%.[16] None of the Saudi patients had ALT level 5 times normal or more. In addition all studies that assessed patients response to lamivudine were among HbeAg positive patients and not pre core mutant virus where other factors may have predictive response to treatment in these patients namely HBV DNA level. Out of the 20 patients who had pre treatment liver biopsy, 7 patients responded to treatment (one patient had mild hepatitis, 5 patients had moderate hepatitis, one patient had severe hepatitis). Though none of the patients with liver cirrhosis responded to treatment, the histological severity did not statistically affect lamivudine response.

Considering HBV dynamics, it is estimated that complete suppression of HBV replication for 12 months with lamivudine would reduce infected cells to 80% of the original population.[17,18] This supports that prolonged treatment with lamivudine is required for HBV eradication. Extended treatment with lamivudine will increase seroconversion to 27% and 33%, after 2 years and 3 years.[19,20] However with prolonged lamivudine treatment, drug resistance increases, with the emergence of tyrosine-methionine-aspartate-aspartate, (YMDD) mutant variants.[20-22] In this study only one patient probably had YMDD variant (increased ALT after initial normalization during treatment). Genotypic analysis for viral isolate from this patient was not possible. This study was designed for one year, however, extending treatment for a longer period may have resulted in a higher response and probably development of more viral mutation.

Tolerability of lamivudine was evaluated by several randomized, double blind studies that showed possibility of increase ALT during and after discontinuation of treatment in addition to fatigue, headache and muscle pain.[9,11,21] One patient (4%) in this study had parathesia which may be drug related with no other identifiable causes and none of the rest had any significant new symptom and this concurs that it is safe and well tolerated.

Combination of alpha interferon with lamivudine in patients with pre-core mutant virus did not significantly improve patient's response;[23] future studies using pegylated interferon in combination with lamivudine or other nucleoside analogues may prove to be more effective.

In conclusion, lamivudine is associated with durable response in patients with precore mutant variant irrespective of the enzymes level and is devoid of significant side effects. Low viral load are associated with a higher response. Despite its effect in HBV, the effect remains modest. Extended treatment for 2 years or 3 years may be required to achieve a higher response, which is associated with higher drug resistance. This needs to be confirmed in future with a larger number of patients and longer treatment duration.

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