# VIROLOGICAL BREAKTHROUGH IN CHRONIC HBV EGYPTIAN PATIENTS RECEIVING LAMIVUDINE THERAPY

## Thesis

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By

***Kamal Hassan Kamal***
(M.B., B.Ch., Cairo University)

Supervisors

**Dr. Gamal Eldin Esmat**
*Professor of Endemic Medicine,*
*Faculty of Medicine, Cairo University*

**Dr. Hanan Abdel Hafez**
*Assistant Professor of Endemic Medicine,*
*Faculty of Medicine, Cairo University*

**Dr. Soheir Ismail**
*Consultant of Endemic Medicine,*
*National Hepatology & Tropical Medicine*
*Research Institute, Egypt*

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بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

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# ABBREVIATIONS

| | |
|---|---|
| **AASLD** | American Association for the Study of Liver Diseases. |
| **AFP** | Alfa-fetoprotein. |
| **ALP** | Alkaline Phosphatase. |
| **ALT** | Alanine Transaminase. |
| **ANOVA** | Analysis of Variance. |
| **Anti-HBe** | Anti Hepatitis B e Antibody. |
| **AST** | Aspartate Transaminase. |
| **cccDNA** | Covalently closed circular DNA. |
| **CDC** | Centers for Disease Control. |
| **CHB** | Chronic hepatitis B. |
| **CTP** | Child–Turcotte–Pugh. |
| **EASL** | European Association for the Study of the Liver. |
| **Eq/mL** | Genome equivalents/ milliliter. |
| **FDA** | Food and Drug Administration. |
| **GGT** | Gamma Glutamyl Transpeptidase. |
| **HAART** | Highly Active Anti-Retroviral Therapy. |
| **HBeAg +ve** | Positive for Hepatitis B e Antigen. |
| **HBeAg –ve** | Negative for Hepatitis B e Antigen. |
| **HBIg** | Hepatitis B Immune globulin. |
| **HBsAb** | Anti Hepatitis B surface Antibody. |
| **HBsAg** | Hepatitis B surface Antigen. |
| **HBV** | Hepatitis B Virus. |
| **HBV DNA** | Hepatitis B Virus deoxyribonucleic acid. |
| **HCC** | Hepatocellular carcinoma. |
| **HCV** | Hepatitis C Virus. |

| | |
|---|---|
| **HDV** | Hepatitis D Virus. |
| **HIV** | Human Immunodeficiency Virus. |
| **IFNs** | Interferons. |
| **IFN-α** | Interferon alfa |
| **IU/ml** | International units IU/ milliliter. |
| **MELD** | Model for End-Stage Liver Disease. |
| **MEq/mL** | Mega-equivalents / milliliter. |
| **NHTMRI** | National Hepatology and Tropical Medicine Research Institute. |
| **NIH** | National Institutes of Health. |
| **NUCs** | Nucleoside / nucleotide analogues. |
| **PCR** | Polymerase Chain Reaction. |
| **Peg IFN-α** | Pegylated Interferon alfa. |
| **RNA** | Ribonucleic acid. |
| **SD** | Standard Deviation. |
| **U/S** | Ultrasound. |
| **ULN** | Upper limit of normal. |

# Abstract

**Background & Aim:** The goal of therapy for hepatitis B is suppression of HBV replication in a sustained manner, thus preventing further progression of the disease. In HBeAg-negative chronic hepatitis B (CHB), Lamivudine is reported to be highly effective in suppressing serum HBV-DNA to undetectable levels by PCR assays, but selection of Lamivudine-resistant mutants is the main concern. In this retrospective study, we aimed at assessing the virological and biochemical responses in HBeAg –ve CHB Egyptian patients receiving Lamivudine therapy.

**Patients & Methods:** Routine laboratory investigations, hepatitis B markers, quantitative HBV-DNA assay by PCR and abdominal U/S were done for 140 HBeAg –ve CHB patients in (NHTMRI) in Cairo who were scheduled for Lamivudine (100 mg /day) (G I: 59 patients for 1 year, G II: 50 patients for 2 years & G III: 31 patients for 3 years).

**Results:** Virological response occurred in76.3%, 72% & 67.7% of cases in G I, II & III respectively. Viral breakthrough rate was 0%, 12% and 25.8% of cases in G I, II & III respectively ($p<0.01$). The mean paired difference between pre and post treatment ALT within each single group was 18.49 ± 30.56, 12.76 ± 33.93 and 27.35 ± 54.10 U/L in group I, II and III respectively ($p< 0.05$ in G II & $p< 0.01$ in G I, III).

**Conclusions:** Lamivudine is highly effective in HBeAg -ve CHB in suppressing serum HBV-DNA to undetectable levels by PCR assays and in ameliorating liver disease. Longer duration of Lamivudine therapy was associated with an increased rate of resistance and hence, viral breakthrough.

**Key words:** HBV, HBeAg –ve, Lamivudine, viral breakthrough, HCC.

# INTRODUCTION

Our understanding of the natural history of hepatitis B virus (HBV) infection and the potential for therapy of the resultant disease has improved ***(EASL, 2012).***

Approximately one third of the world's population has serological evidence of past or present infection with HBV ***(Lok et al., 2007).*** An estimated 350 million persons worldwide are chronically infected with HBV ***(Lavanchy, 2004).*** The global prevalence of HBsAg varies greatly and countries can be defined as having a high, intermediate and low prevalence of HBV infection based on a prevalence of HBsAg carriers of >8%, 2:7%, and <2% respectively***(McMahon, 2005).*** In developed countries, the prevalence is higher among those who immigrated from high or intermediate prevalence countries and in those with high risk behaviors ***(Mast et al., 2005).*** Studies in the Middle East show the prevalence of HBsAg to range from 3% to 11% in Egypt ***(Qirbi et al., 2001).*** A decrease in HBV incidence is expected among children in intermediate-endemicity countries (3:5% HBsAg prevalence), such as Egypt, where 90% immunization coverage has been achieved ***(Zakaria et al., 2007).***

HBV is transmitted by perinatal, percutaneous, and sexual exposure, as well as by close person-to-person contact presumably by open cuts and sores, especially among children in hyperendemic areas ***(Mast et al., 2005).***

The spectrum of disease and natural history of chronic HBV infection is diverse and variable, ranging from a low viremic inactive carrier state to progressive chronic hepatitis, which may evolve to

cirrhosis and hepatocellular carcinoma (HCC) ***(Ganem and Prince, 2004).***

Carriers of HBV are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC). Although most carriers will not develop hepatic complications from chronic hepatitis B, 15% to 40% will develop serious sequelae during their lifetime ***(Bosch et al., 2005).*** HBV-related end stage liver disease or HCC are responsible for over 1 million deaths per year and currently represent 5–10% of cases of liver transplantation ***(Hoofnagle et al., 2007).***

CHB may present either as HBeAgpositive or HBeAg negative CHB. HBeAg positive CHB is due to so-called ‘‘wild type” HBV. It typically represents the early phase of chronic HBV infection. HBeAg-negative CHB is due to replication of naturally occurring HBV variants with nucleotide substitutions in the precore and/or basic core promoter regions of the genome and represents a later phase of chronic HBV infection ***(Rizzetto et al., 2008).***

The prevalence of the HBeAg negative form of the disease has been increasing over the last decade as a result of HBV-infected population aging and represents the majority of cases in many areas, including Europe ***(Zarski et al., 2006).***

The precore and core mutant variants of chronic HBV (HBeAg negative CHB) are commonly found in association with genotype D ***(Funk et al., 2002).*** In Egypt, HBeAg-negative CHB accounts for more than 80% among older age group ***(El-Zayadi, 2007).***

The aim of treatment of chronic hepatitis B is to achieve sustained suppression of HBV replication and remission of liver disease. The ultimate goal is to prevent cirrhosis, hepatic failure and HCC. Parameters used to assess treatment response include normalization of serum ALT, decrease in serum HBV DNA level, loss of HBeAg with or without detection of anti-HBe, and improvement in liver histology. Approved Antiviral Therapies for CHB patients include Standard IFN, Peg IFN& Nucleoside/Nucleotide analogues (NUCs) including Lamivudine, Adefovir dipivoxil, Tenofovir, Entecavir& Telbivudine ***(Hoofnagle, 2007).*** A major concern with long-term NUCs treatment is the occurrence of antiviral-resistant mutations. The rate at which resistant mutants occur is related to pretreatment serum HBV DNA level, rapidity of viral suppression, duration of treatment, and prior exposure to NUCs therapies ***(Bartholomeusz and Locarnini, 2006).***

Virologic breakthrough is usually followed by biochemical breakthrough, which is defined as elevation in ALT during treatment in a patient who had achieved initial response. Emergence of antiviral-resistant mutations can lead to negation of the initial response, and in some cases hepatitis flares and hepatic decompensation. Antiviral-resistant mutations can be detected months and sometimes years before biochemical breakthrough ***(Fung et al., 2006).*** Thus, early detection and intervention can prevent hepatitis flares and hepatic decompensation, and this is particularly important in patients who are immunosuppressed and those with underlying cirrhosis. Another potential consequence of antiviral-resistant mutations is cross resistance with other NUCs, thus limiting future treatment options ***(Yim et al., 2006).***

Follow-up of patients receiving continued Lamivudine treatment showed that the rates of maintained virologic and biochemical response decreased with time due to drug-resistant mutants ***(Papatheodoridis et al., 2005)***. In patients with maintained viral suppression, necroinflammation is reduced and decrease in fibrosis score as well as regression of cirrhosis was observed ***(Dienstag et al. b, 2003).*** However, histologic benefit was negated among patients with breakthrough infection. Several studies reported that patients with maintained viral suppression had lower rates of hepatic decompensation as well as liver-related mortality ***(Papatheodoridis et al., 2005).***

# AIM OF THE WORK

The aim of our study was to assess the virological and biochemical responses as well as breakthrough in HBeAg – negative chronic hepatitis B Egyptian patients receiving Lamivudine therapy.

# Epidemiology

Hepatitis B virus (HBV) infection is a global health problem. Approximately one third of the world's population has serological evidence of past or present infection with HBV and 350-400 million people are chronically infected ***(EASL, 2012).*** The spectrum of disease and natural history of chronic HBV infection is diverse and variable ***(Hoofnagle et al., 2007).***

Approximately 75% of chronically infected patients reside in Asia and the Western Pacific ***(Lavanchy, 2004).*** In the United States, there are an estimated 1.25 million hepatitis B carriers, defined as persons positive for hepatitis B surface antigen (HBsAg) for more than 6 months ***(Mast et al., 2006).***

The prevalence of HBsAg in Egypt is of intermediate endemicity (2–8%). Nearly 2-3 million Egyptians are chronic carriers of HBV. In Egypt, it appears that HBV transmission is a mixture of perinatal and horizontal transmission. However, the majority of HBV infection is acquired by horizontal transmission ***(El-Zayadi, 2007).***

Carriers of HBV are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) ***(Lok and McMahon, 2009).*** Although most carriers will not develop hepatic complications from chronic hepatitis B, 15% to 40% will develop serious sequelae during their lifetime ***(Bosch et al., 2005).*** Worldwide, chronic HBV infection is the most common cause of HCC. More than 50% of HCC cases worldwide and 70-80% of HCC cases in highly HBV endemic regions are attributable to HBV ***(Yeung et al., 2005).*** Each year around 1.2 million die of HBV related chronic liver disease ***(Lai, 2004).***