# PERIOD PREVALENCE AND CUMULATIVE INCIDENCE OF LAMIVUDINE RESISTANCE AND ITS ASSOCIATED FACTOR IN CHRONIC HEPATITIS B PATIENT IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

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| | |
|---|---|
| **CHB** | Chronic Hepatitis B |
| **HBV** | Hepatitis B Virus |
| **DNA** | Deoxyribonucleic Acid |
| **HBsAg** | Hepatitis B surface antigen |
| **cccDNA** | closely covalent circular DNA |
| **mRNA** | messenger ribonucleic acid |
| **ER** | Endoplasmic reticulum |
| **HBeAg** | Hepatitis B envelop antigen |
| **Anti HBe** | Hepatitis B e antibody |
| **HIV** | Human Immunodeficiency Virus |
| **ALT** | Alanine aminotransferase |
| **AST** | Aspartate aminotransferase |
| **YMDD** | Amino acid sequence of thyrosine-methionine-aspartate-aspartate |
| **EASL** | European Association for the Study of the Liver |
| **APASL** | Asian Pacific Association for the Study of the Liver |
| **PCR** | Polymerase Chain Reaction |
| **AASLD** | American Association for the Study of Liver Disease |
| **CPG** | Clinical Practice Guidelines |
| **HUSM** | Hospital Universiti Sains Malaysia |

## ABSTRAK

*Tajuk:*

Perkadaran kerintangan Hepatitis B virus terhadap lamivudine dan penentuan faktor-faktor terjadinya kerintangan tersebut di kalangan pesakit hepatitis B kronik yang mengamalkan pengambilan lamivudine dalam jangka masa panjang sebagai rawatan tunggal.

*Latar Belakang:*

Lamivudine merupakan sejenis anti-virus yang berkesan dalam merawat hepatitis B kronik. Walau bagaimanapun, terdapat risiko di mana virus hepatitis B bermutasi dan ini menyebabkan kewujudan rintangan terhadap lamivudine. Kerintangan virus hepatitis B terhadap lamivudine menyebabkan ianya tidak lagi berkesan dan sekaligus kadar pembiakan virus akan meningkat semula dan disusuli pula dengan peningkatan ujian-ujian fungsi hati yang berkaitan. Walaupun terdapat alternatif anti-virus lain yang lebih berkesan sebagai rawatan pendahuluan seperti entecavir dan tenofovir, terdapat masalah tertentu seperti harganya yang mahal yang menyebabkan anti-virus ini tidak terdapat di semua pusat rawatan.

*Objektif*

Tujuan kajian ini adalah untuk menentukan kadar terjadinya kerintangan virus hepatitis B terhadap lamivudine di kalangan pesakit di HUSM, yang mana pengambilannya dimulakan di antara tahun 2010 sehingga 2016. Selain itu, kajian ini ingin menentukan sekiranya terdapat faktor sehingga wujud kerintangan terhadap lamivudine. Dan yang terakhir, kajian ini bertujuan untuk menentukan kadar keberkasanan rawatan lamivudine di kalangan pesakit hepatitis B di HUSM.

*Metodologi*

Ini merupakan kajian retrospektif ke atas 41 pengidap hepatitis B kronik yang diberi rawatan lamivudine dari tempoh 2010-2016. Rekod perubatan telah diteliti dan maklumat-maklumat yang relevan telah dicatat ke dalam perisian komputer statistik. Subjek yang telah mendapat kerintangan virus terhadap lamivudine dan faktor yang berkaitan kemudiannya dianalisa.

*Keputusan*

Daripada kesemua 41 pesakit yang telah diteliti rekod perubatannya, sebanyak 9 pesakit mendapat kerintangan terhadap lamivudine. Ini manjadikan prevalansi semasa tempoh 6 tahun tersebut adalah 21.9%. Peratusan kes kerintangan lamivudine pada tahun pertama, kedua dan ketiga adalah masing-masing sebanyak 4.9%, 12.2% and 21.9%. Faktor seperti umur, jantina, kaum, kuantiti HBV DNA sebelum rawatan, ALT dan tempoh panjang rawatan adalah tidak signifikan dalam menentukan terjadinya kerintangan terhadap lamivudine. Kajian ini juga menunjukkan kadar keberkasanan lamivudine dalam merawat Hepatitis B sebanyak 85.9%.

*Kesimpulan*

Kadar kekerapan berlakunya kerintangan lamivudine di kalangan pengidap hepatitis B kronik yang mengamalkan pengambilan lamivudine sebagai rawatan tunggal dalam jangka masa panjang di dalam kajian ini adalah lebih rendah berbanding dengan yang dilaporkan di dalam kajian-kajian lain sebelum ini. Walau bagaimanapun, untuk membuat sebarang cadangan dalam pemilihan anti-virus, kajian lanjut dengan lebih banyak subjek adalah perlu.

## ABSTRACT

*Title:*

Period Prevalence of Lamivudine Resistance and Its Associated Risk Factor in Chronic Hepatitis B Patient Receiving Long Term Lamivudine Monotherapy in Hospital Universiti Sains Malaysia (HUSM)

*Background:*

Lamivudine is an established treatment options for chronic hepatitis B (CHB), however development of viral resistance followed by virological breakthrough made it a less favourable as a first line treatment option. While the alternatives such as entecavir and tenofovir are favoured for first line options due to its higher genetic barrier to resistance, constraints such as financial issue may not make these drugs readily available in some practice.

*Objectives:*

The aim of the study is to established period prevalence of lamivudine resistance among patient with CHB who received Lamivudine from January 2010 to Dec 2016, and to explore if there are any factors that associate with development of resistance. The study also aims to look at the response rate for those treated with lamivudine during the same period.

*Methods*

This is a retrospective record review done by reviewing record of 41 patients who was or on lamivudine during the period of 2010 to 2016. Subjects are CHB patient who are treated with lamivudine as a first line monotherapy. All relevant data are gathered from the review of case note and recorded in statistical software for analysis.

*Results*

Total number of patients included in this study is 41. During the 6 years periods, 9 patients with lamivudine resistance were detected and this yields an overall period prevalence of 21.9%. The cumulative incidence of developing resistance during first year, second year and third year while on lamivudine is 4.9%, 12.2% and 21.9% respectively. Factors such as age, gender, race, pre-treatment viral load, serum ALT, and duration of treatment are shown not associated with development of resistance. In terms of response to treatment, this study shows an overall response rate of 89.5%.

*Conclusion*

The prevalence and incidence of lamivudine resistance in this study is lower in comparison with the prevalence and cumulative incidence cited in many literatures. However, further study is warranted to include more patients from other centres in order to improve power of this study, before any recommendation can be proposed.

# CHAPTER 1: INTRODUCTION

## 1.3 Epidemiology of Adult Hepatitis B Virus Infection

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the family of hepadnaviruses. It is estimated that some 240 million people worldwide have chronic HBV infection with Africa and Asia being among the regions with the highest prevalence (Sarin *et al.,* 2016). The global prevalence of Hepatitis B infection varies greatly and can be categorized into high, intermediate and low prevalence country base on HBsAg positivity of >8%, 2-7% and <2% respectively (McMahon, 2005).

About one million Malaysians have chronic HBV infection and Hepatitis B contributes to 75% of all viral hepatitis cases in Malaysia with male to female ratio of 2:1. Malaysia is a country under intermediate prevalent country (Raihan, 2016). The seroprevalance for HBV surface antigen in Malaysia is estimated around 5%. Figure 1 shows prevalence of HBV infection trend in Malaysia. From 1990 to 1997, it shows that there is steadily decrease trend until sharp rises recorded in 1998. This rises is due to the detection from mandatory hepatitis screening for all foreign worker. The decrease trend followed after 2000 before it increase again in 2010 due to the rule implemented by the government that all cases of hepatitis B to be reported to hospitals (Raihan, 2016). The rate of HBsAg detection in school children was reduced from 2.5% (for those who born in 1985) to 0.4% (for those who born in 1996) due to introduction of childhood Hepatitis B vaccination (Raihan, 2016) .Vertical transmission is responsible for most of the HBV carrier due to the high viral load in Malaysian child-bearing woman (Raihan, 2016).

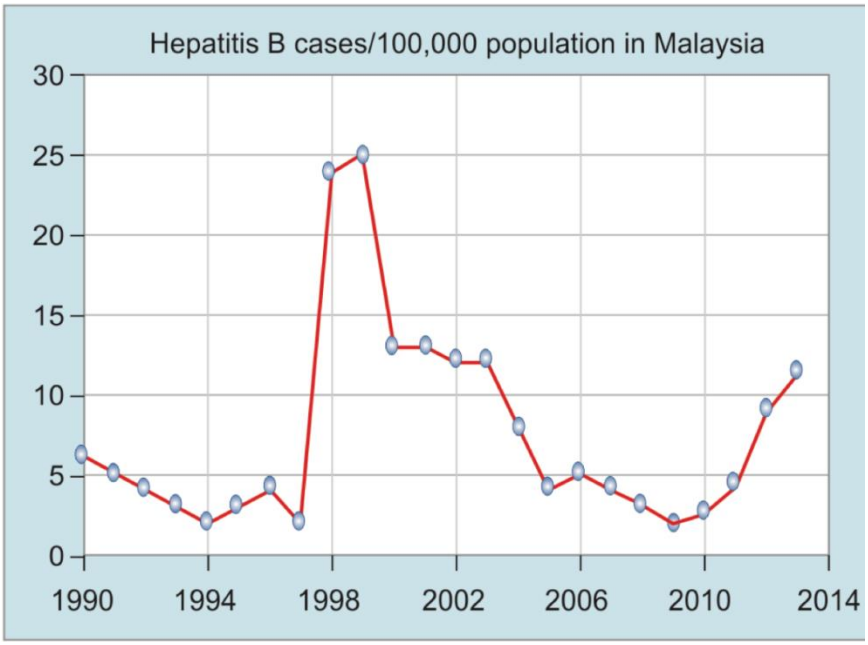


**Figure 1: The trend of Hepatitis B prevalence in Malaysia from 1990-2014 (*Adapted from Raihan 2016*)**

## 1.4 Hepatitis B Virus Infection in Human

### 1.2.1 Structure of the Hepatitis B Virus

HBV is small, the size of the circulating virion measured at 42-nm Dane particle which is comprises of 2 parts - the nucleocapsid and its circulating enveloped. The nucleocapsid contains about 3.2 kilobase genome, as a partially double stranded DNA. This compact genome encoded for 7 types of viral protein – 3 envelop proteins, the nucleocapsid core protein, the secretory Hepatitis B e antigen, the viral reverse transcriptase/polymerase and the X protein (Ghany and Liang, 2007). The organization of the HBV genome is illustrated in the Figure 2.

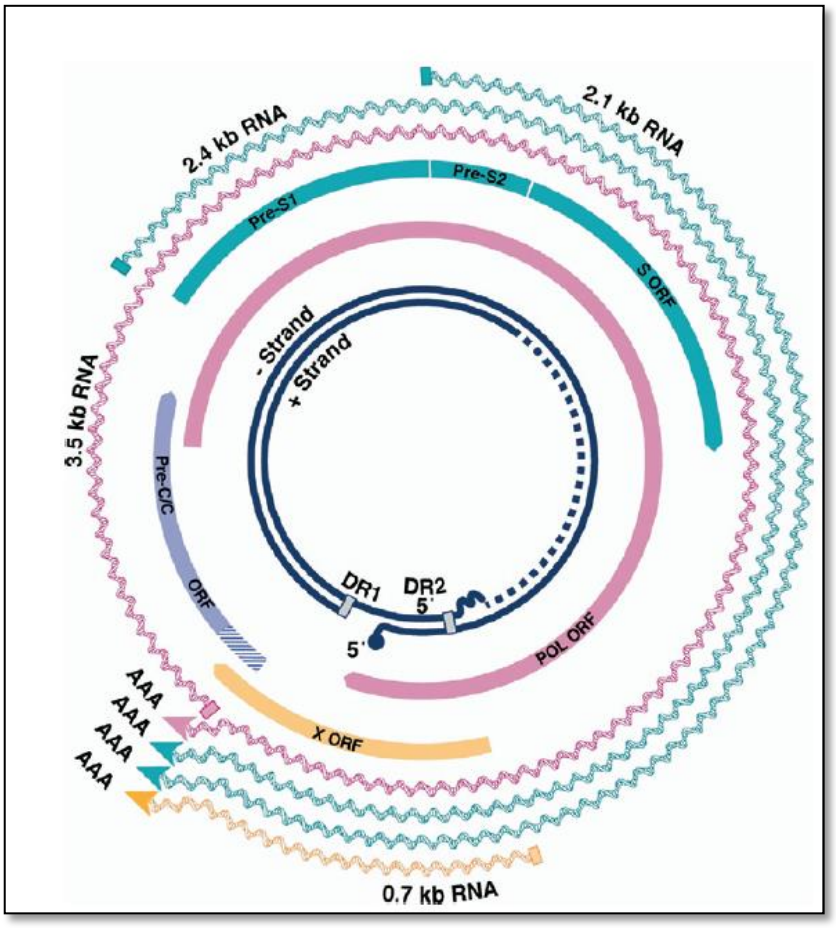


**Figure 2: Organization of HBV Genome**
**(*Adapted from Marc Ghany April 2007 DOI:*10.1053/j.gastro.2007.02.039*)***

Referring to Figure 2 above, the *inner circle* depicts the HBV genome encoded for polymerase (*dark circle*). It attached to the 5' end of the minus strand and a capped oligoribonucleotide (*corkscrew*) attached to the 5' end of the incomplete plus strand. Two, 11-base pair direct repeats located at the 5' end of the minus and plus strands - *DR 1* and *DR 2*, are important for strand-specific synthesis. The 4 open reading frames are shown between the inner and outer circles. These consist of the pre-C/core genes, the polymerase gene, the pre-S1, pre-S2 and S genes, and the X gene. Lastly, the *outer circle* shows the 4 major viral mRNAs -the 3.5-kilobase (kb) core or pregenomic mRNA, the 2.4-kb pre-S1 mRNA, 2.1-kb pre-S2/S mRNA, and the 0.7 kb X mRNA. All mRNAs share a common poly-A 3' end (Ghany and Liang, 2007).

### 1.2.2 Replication Cycle of the HBV

In human host, the HBV replication cycle predominantly takes place in the hepatocytes. There is still a debate whether this replication also occurs outside hepatocytes. The mechanism in which the HBV attach and enter the hepatocyte is still not completely understood. After gained entry into hepatocytes and in the cytoplasm, the outer envelope disintegrates. This will release the viral genomes which then make its way toward and into host's nucleus. In the nucleus, a fully double stranded closely covalent circular DNA (cccDNA) is produce after the initial circular genome underwent a repair process, which mediated by host and viral polymerase enzymes. The cccDNA now is ready and now becomes a main template for the production of all the viral messenger RNA (mRNA) which are then transport out of nucleus into cytoplasm again at which the translation of viral proteins, nucleocapsid assembly and viral replication occurs. This viral mRNA strands can be divided into pregenomic mRNA and subgenomic mRNA. Pregenomic mRNA is encoded for reverse transcriptase, the core and polymerase enzymes. Subgenomic mRNA is necessary for the translation of the envelope protein and the X protein.

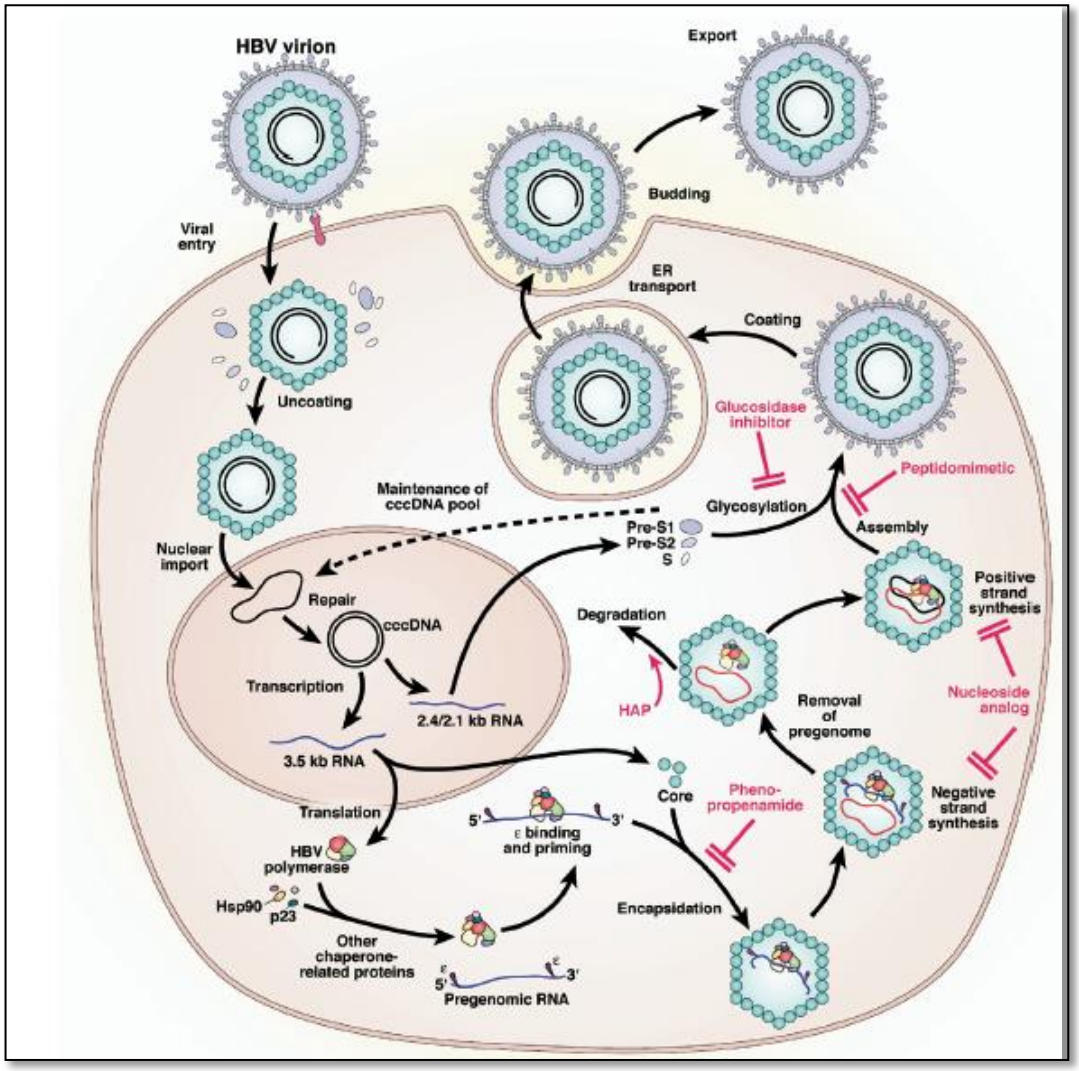


**Figure 3: Replication cycle of HBV and molecular target of HBV replication**

**(*Adapted from Marc Ghany April 2007 DOI:*10.1053/j.gastro.2007.02.039*)***

Figure 3 illustrates the complex interaction that ultimately leads to encapsidation of the pregenomic RNA which takes place in cytoplasm. This complex interaction is consists of interplay in between the epsilon, core viral particles, HBV polymerase, and various chaperones protein. The generation of negative and then positive DNA strands occurs within the viral nucleocapsid and reverse transcriptase is essential in this process. Viral assembly takes place in the endoplasmic reticulum (ER). 'Mature' nucleoside is then finally coats with envelope protein before budding and release of virion into the blood stream (Ghany and Liang, 2007).

### 1.2.3 Natural history of HBV infection in Human

In understanding the natural history of chronic HBV infection, it is important to acknowledge that the disease phases that follows reflects a complex interactions between the level of HBV replication activity and the host immune reactivity against the replicating HBV. The four phases are *immune-tolerance* phase, *immune-clearance* phase, low replicative phase and *reactivation* phase. Transition from one phase of chronicity to the next is not recognizable in all patients, either because it may not be obligatory step in the overall natural course of the infection, or because it is of very short duration to allow it to be clinically manifested. The transition from one phase to the next along its natural history course is largely depends on the age at which the virus is acquired. For example, patient who acquire HBV infection either at birth or within first 1-2 years of life, they typically will have a prolonged immune tolerance phase followed by equally prolonged immune clearance phase (Sarin *et al.*, 2016).

In the other hand, patient who acquire the virus after early childhood generally do not have the immune tolerant phase, but likely to go straight into immune clearance phase (characterized by HBeAg seroconversion to anti-HBe and HBV DNA viral load is relatively low level or undetectable) and followed by relatively quiet disease after that. In individual with active replication and high viral load burden, damaged hepatocytes may progress into cirrhosis and liver failure, and eventually development of hepatocellular carcinoma. While majority of chronically infected HBV patient will not develop adverse liver outcome, still 15-40% of them will suffered from its complication during lifetime (Sarin *et al.*, 2016).

### 1.3 Effect of Vaccination in the Asia Pacific Region

Introduction of Hepatitis B vaccination in newborn has changed the epidemiological landscape of Hepatitis B infection. World Health Organisation (WHO) has published a report in 2012 demonstrating a robust data that the prevalence of chronic Hepatitis B is decreasing in trend from 1990 to 2005 in the most region of the world (Ott *et al.*, 2012). In mainland China, the prevalence of HBsAg positivity among general population decrease from 9.75% in 1995 to 7.18% in 2006. But the dramatic changes is seen in the subset of children less 5 year old, from 9.67% in 1992 to 0.96% in 2006 (Liang *et al.*, 2009). Data from Korean studies shows similar encouraging decreasing trend of HBsAg prevalence among their teenagers (age 10 to 19 year old), from 2.2% in 1998 to 0.12% in 2010 (Kim *et al.*, 2013).

### 1.4 Lamivudine as Monotherapy in HBV infection

The goal of Hepatitis B treatment is to suppress viral replication before there is irreversible damage to the liver. There are few antiviral agents that are effective in achieving this suppression. One of them is lamivudine (molecular formula $C_8H_{11}N_3O_3S$), a type of nucleoside analogue reverse transcriptase inhibitor. Lamivudine is an enantiomer of 2', 3'-dideoxy 3'-thiacytidine (ß-L-(-)-2', 3'-dideoxy 3'-thiacytidine) which originally developed for the treatment of Human Immunodeficiency Virus (HIV) infection. Subsequently it is also discovered that lamivudine also effective against HBV infection, irrespective of the HBeAg status as clinical trial has proved its effectiveness in both populations (Lai *et al.*, 1998; Tassopoulos *et al.*, 1999).

The mechanism behind lamivudine's ability to inhibit viral replication is via its ability to inhibit the DNA polymerase action and DNA replication by targeting the HBV-encoded reverse transcriptase. This leads to interference with DNA synthesis which will eventually cause DNA chain termination. Before the era of nucleoside analogue, treatment of chronic HBV infection is limited to cytokine therapy such as alpha interferon. Treatment of HBV infection with nucleoside analogue is much preferable compared to interferon due to its convenience (oral form) and favourable side effects profile. Nucleoside analogue also reduce the HBV DNA viral load faster than the interferon. Treatment of Hepatitis B with lamivudine 100mg daily for 52 weeks is associated with marked suppression of HBV DNA levels with a median reduction of 95%-99% throughout the treatment period in two large phase III trials (Dienstag *et al.*, 1995; Dienstag *et al.*, 1999). One of the earliest efficacy data of lamivudine against chronic Hepatitis B infection in Asian population is published in 1998 by Lai et al. In this randomized control trial, use of lamivudine 100mg daily for 1 year is associated with higher rate of HBeAg seroconversion, undetectable HBV Viral DNA, sustained normalization of ALT compared to placebo group (Lai *et al.*, 1998). In 2003, Lok et al demonstrated that the efficacy of lamivudine is maintained for treatment of HBeAg positive Hepatitis B infection for up to 6 years and it has an excellent safety profile (Lok *et al.*, 2003). Other study demonstrated that HBV suppression is maintained in patient on lamivudine for at least 10 years (Sun *et al.*, 2011). In a nutshell, lamivudine is efficacious in treatment of HBV infection as measured by biochemical (ALT), serological, virological and histological outcome.

## 1.5 HBV Resistance to Lamivudine

While nucleoside analogues is effective and have great safety profile in the treatment of chronic HBV infection, it must be used in prolonged duration in order to sustain viral suppression. This is due to high rate of virological relapse if the treatment ceased. A few studies has shown that once the nucleoside analogue was discontinued, HBV DNA levels will return to baseline in most patients with the exception of those who achieve a sustained HBeAg seroconversion (Dienstag *et al.*, 1995; Dienstag *et al.*, 1999; Lai *et al.*, 1998). The virological relapsed is caused by development of antiviral drug resistance, which associated with long-term therapy. Serious consequences may occur in those with resistance as rebound virological replication may potentially leads to hepatitis flares and death.

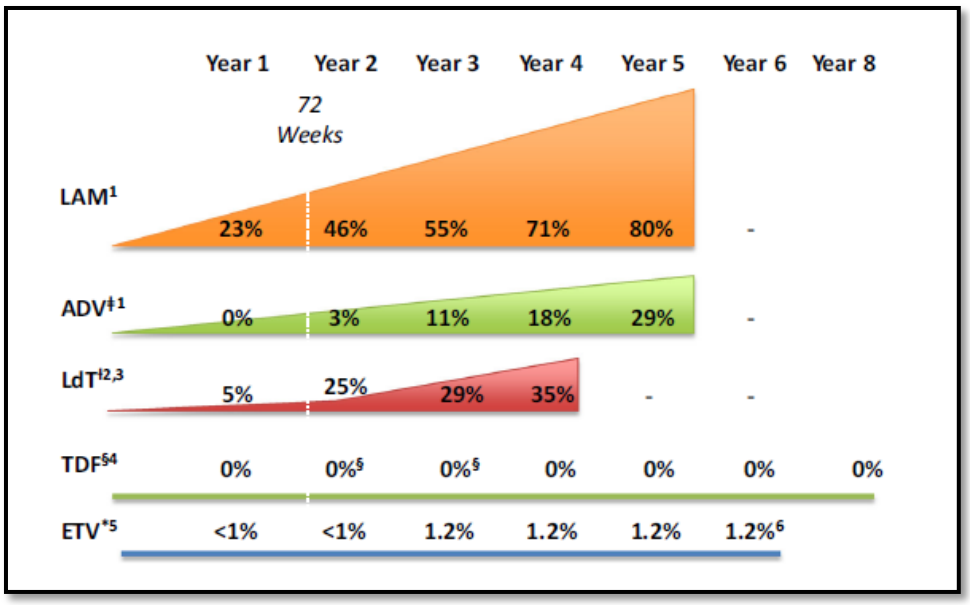


**Figure 4: The cumulative incidence of lamivudine resistance in comparison with other anti-viral** *(Adapted from Sarin 2016)*

Lamivudine is potent, but also has the lowest genetic barrier amongst all the nucleoside analogues for HBV. Few publications which studied the HBV resistance rates towards lamivudine have reported varied figures for prevalence and cumulative incidence. Figure 4 illustrates the cumulative incidence of lamivudine resistance and other antiviral for HBV. It shows that lamivudine has highest incidence of resistance and entecavir is the only nucleoside analogue without any associated clinical resistance.

### 1.5.1 Definitions of Lamivudine Resistance

Lamivudine resistance can be defined in several ways. Genotypic resistance is defined as detection of mutation(s) in the HBV genome that are known to confer resistance and developed during lamivudine therapy (Sarin *et al.*, 2016). Phenotypically, it is defined as decreased susceptibility (in vitro testing) to inhibition by lamivudine (Sarin *et al.*, 2016). Lamivudine resistance in HBV infection is clinically suspected whenever there is a virological breakthrough, which is defined as increase of serum HBV DNA > 1 log IU/ml from nadir of initial response during therapy (Sarin *et al.*, 2016). In clinical practice, due to cost associated with measurement of HBV DNA viral load, measurement of liver enzyme (particularly serum aminotransferase, ALT) is more readily available and measured more frequently along the course of lamivudine therapy. Acute flare of hepatitis in HBV patient is defined as elevations of ALT more than twice the baseline value or more than five times the upper limit value (Kumar *et al.*, 2006). This hepatitis flare when associated with development of lamivudine resistance is termed biochemical breakthrough (Jang *et al.*, 2005). Virological breakthrough theoretically must preceded the biochemical breakthrough, but in practice this is not always possible to recognized as ALT is more readily and more frequently measured compared to HBV DNA viral load.

### 1.5.2 Mechanism of Lamivudine resistance in HBV

The mechanism that underlies lamivudine resistance is related to how lamivudine works in suppressing viral replication. Lamivudine targets the HBV polymerase by interfering with its function and due to this interference, it leads to newly synthesized DNA chain termination. The HBV polymerase is a multifunctional viral protein and it has a pivotal role during viral replication process. It comprises of four domains, namely a priming domain, a spacer domain, a catalytic domain and a terminal carboxy region that has ribonuclease H activity. The catalytic domain serve as RNA-dependent RNA polymerase/DNA polymerase (Ghany and Liang, 2007). The sequence structure of the HBV polymerase is illustrated in figure 4.

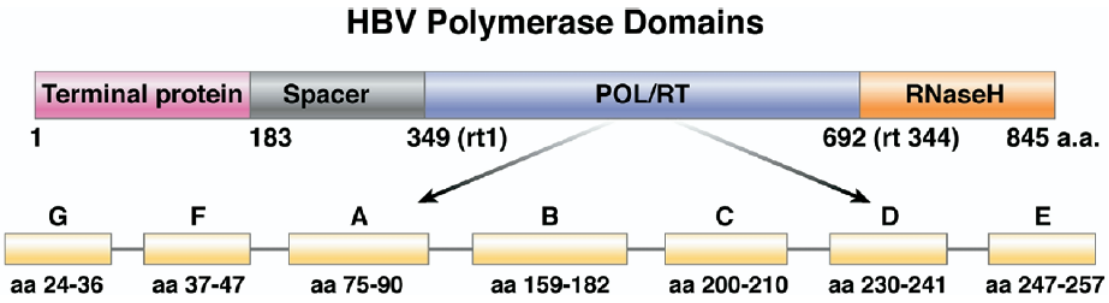


**Figure 5: HBV polymerase domains**

***(Adapted from Ghany April 2007 DOI:*10.1053/j.gastro.2007.02.039*)***

As demonstrated in figure 5, the catalytic domain can be further divided into 7 subdomains. Subdomain C contains a sequence of 4 amino acids: tyrosine, methionine, aspartate, aspartate (YMDD). This small subdomain is actually highly conserved among viral polymerase/reverse transcriptase. It binds 2 magnesium ions and represents the active site of the enzymes.

HBV has high replication rate with estimation of $10^{12}$ virions being produced per day. The mutation rate is approximately $10^{-5}$ substitution/base/cycle. In individual with an active replication, this mutation rate translates into approximately $10^{10\text{-}11}$ point mutation produced per day. This means that in a relatively small genome virion like HBV (~3.2 kilobase pair), all possible single base changes can be produce in just a day.

There are few principal mutations which contribute to the lamivudine resistance. Substitution of methionine to valine or isoleucine (rtM204V/I) in the YMDD motif of the catalytic domain of HBV polymerase is the commonest one. The rtM204V/I is usually associated with the compensatory rtL180M mutation especially in HBV genotype A, B and C (Damerow *et al.*, 2010). This mutation leads to changes in the DNA polymerase conformational structure which eventually leads to: (1) Steric hindrance to decrease binding of lamivudine to the viral polymerase and (2) reduced catalytic activity to incorporate lamivudine triphosphate into replicating viral DNA.

The rtM204V/I mutation is not unique to lamivudine. It is also a mechanism behind resistance to other L-nucleoside analogue such as telbivudine, but agent like entecavir and tenofovir will remain sensitive in those mutant viruses. In vitro study by Allen MI demonstrated that the rtM204V/I mutations confer up to 10000 fold resistance suggesting that the antiviral response is not restored with lamivudine dose increment(Allen *et al.*, 1998). Other mutations that confer lamivudine resistance in HBV are rtL80V/I, rtV173L, rtL180M, rtA181V/T, rtT184S, rtQ215S (Ghany and Doo, 2009; Stuyver *et al.*, 2001). Recent studies suggest that the pattern of mutation is related to the HBV genotype. HBV-A favours rtM204V, while HBV B – D prefers rtM204I (Mirandola *et al.*, 2012).

### 1.5.3 Contributing Factors to Development of Anti-Viral Resistance

Development of resistant towards antiviral can be due to many factors. 2 of the most important one are viral fitness and the potential genetic barrier resistance of the anti-viral. Viral fitness is defined as the ability of virus to replicate in defined environment and this ability is depends on the replication activity and replication space. Replication activity refers to the ability of mutant virus to replicate in the absence of a drug compared to the wild-type, drug-sensitive virus. In the other hand, replication space refers to the ability of the environment to support replication (hepatocyte in the case of HBV). In the case of HBV mutant virus, although the replication fitness was initially less, but in the environment where there is a presence of an antiviral agent, their population has an advantage and will thrive. Over time, compensatory mutations occur. Compensatory mutation refers to secondary mutations that occurs which will restores functional defects in the viral polymerase caused by primary mutations. It empowers the mutant virus to replicate at near wild type level, leading to development of antiviral resistance.

Genetic barrier to resistance can be regarded as the number of mutations that the virus must accumulate before it can replicate effectively in the presence of antiviral. A drug with high genetic barrier will have a lower likelihood of resistance because it requires a high accumulation of mutations before it becomes ineffective. This antiviral property is depends on the structure of the anti-viral compound.

Host factor also plays significant role in the development of resistant to anti-viral. Viral replication rate may be different in immunosuppressed individual, thus this potentially can affects the rate of mutations development. Ability to achieve effective

drug concentrations quickly is also crucial as the potency of a drug is reflected by how rapidly it can suppress viral replication; the more rapid, the lower the risk of developing antiviral resistance. This is not always easy to achieve especially in obese patient, or those who are not compliant to therapy.

### 1.5.4 Clinical Significance of Lamivudine Resistance in HBV

The incidence of lamivudine resistant has been evaluated in several studies. Table 1 below summarizes the cumulative incidence quoted by European Association for the Study of the Liver (EASL) and Asian Pacific Association for the Study of the Liver (APSL) in their HBV infection Clinical Practice Guidelines. It highlights that the percentage is higher with prolonged duration of the therapy ("EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection," 2017).

| **Duration on Lamivudine** | **Cumulative Incidence** | |
|---|---|---|
| | EASL 2017 | APASL 2016 |
| **1 year** | 24% | 23% |
| **2 year** | 38% | 46% |
| **3 year** | 49% | 55% |
| **4 year** | 67% | 71% |
| **5 year** | 70% | 80% |

**Table 1: Cumulative incidence of Lamivudine Resistance**

Study by Liaw et al demonstrated that resistance to lamivudine among the HBeAg positive chronic hepatitis B patient on lamivudine is 24% (81 of 335) during the clinical trial (Liaw *et al.*, 2000). In HBeAg negative population, Osman et al demonstrated that

overall virological breakthrough rate of 35% (Osman, 2011). Lok et al shows that the rate of lamivudine resistance is increase with longer duration of therapy, showing percentage of patient with rtM204V/I mutation of 19%, 23%, 62%, 76% and 80% at <1year, 1-2 year, 2-3 year, 3-4 year and 4-5 year respectively (Lok *et al.*, 2003).

Diagnosing HBV resistance against lamivudine can be done genotypically by detecting YMDD variants by means of polymerase chain reaction (PCR). The test will recognize if the mutation is present or not and also a specific mutation subtype. However this is rarely done in clinical practice. Virological breakthrough, and biochemical breakthrough is clinically more feasible way to detect presence of resistance.

## 1.6 Rationale of Study

Data on HBV resistance to lamivudine have a significant impact on the many HBV infection treatment guidelines. Recently updated Asia Pacific Association Study of the Liver (APASL) Clinical Practice Guidelines on the management of hepatitis B in 2016 have proposed tenofovir and entecavir as a first line agent in treatment naïve individual (Sarin *et al.*, 2016). This highlights that the role of lamivudine as first line monotherapy is fading. Similarly, lamivudine is no longer first line antiviral for both American Association for the Study of Liver Disease (AASLD) and European Association for the Study of the Liver (EASL) ("EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection," 2017; Terrault *et al.*, 2016).

While entecavir or tenofovir is the preferred first line agent due to its superior clinical efficacy and safety profile compared to lamivudine, the associated extra cost that comes with it may not make it readily available for all patients. This is exceptionally true in current challenging economic climate where a tight financial resource is common including healthcare budget cut. APASL acknowledge in their CPG in 2016 that lower cost for lamivudine therapy is one of the reason why it is still a commonly used therapy in many of Asian country. Lamivudine is an inexpensive drug and is still clinically relevant especially in a population where the rate of resistance is known to be low.

In this study, we aimed to establish lamivudine resistance rate in our cohort of HBV patient. Based on our clinical experience, we observe that our resistance rate is lower compared with what was being reported in the literature. If the lower resistance rate is observed in our centre, this is a big supporting point to the use of lamivudine as a first line therapy for treatment of chronic HBV infection.

The proportion of lamivudine resistant case in this study possibly can be reported in term of prevalence and period prevalence. Period prevalence takes account a defined period of time in its denominator, as compared to prevalence where is just at certain point of time. This study involves a specified period of time of 6 years from 2010-2016, so period prevalence was deemed more appropriate measure in this study.

# CHAPTER 2: RESEARCH OBJECTIVE

## 2.1 General Objective

The aim of the study was to established period prevalence of lamivudine resistance among patient with CHB who received lamivudine from January 2010 to December 2016, and to explore if there were any factors associated with development of resistance. The study also aimed to look at the response for those with CHB during the same period.

## 2.2 Specific Objective:

- To determine the period prevalence and cumulative incidence of lamivudine resistance in chronic hepatitis B patient receiving long term lamivudine in HUSM from 2010-2016.
- To determine the associated factor for development of lamivudine resistance
- To determine the period prevalence of patient with biochemical, serological or virological response among chronic hepatitis B patients receiving long term lamivudine in HUSM from 2010-2016

# CHAPTER 3: METHODOLOGY

## 3.1 Research Design

This is retrospective record review involving patient with CHB receiving lamivudine in HUSM Gastroenterology Clinic from 2010-2016

## 3.2 Study Location

Gastroenterology Clinic HUSM and Record Office HUSM

## 3.3 Study Duration

January 2017 – Nov 2017

## 3.4 Study Approval

This study was approved by the Research and Ethic Committee, Universiti Sains Malaysia with JEPeM study protocol code USM/JEPeM/17080363

## 3.5 Reference Population

Patient who were diagnosed with HBV infection, antiviral naive and received lamivudine as first line monotherapy in HUSM from year January 2010 to December 2016.

## 3.6 Sampling Method

List of all patients who was prescribed lamivudine from 2010-2016 were extracted by using electronic pharmacy record. Their medical records were then traced from record office and examined for inclusion

and exclusion criteria and those who were eligible, all the necessary information is extracted based on proforma (Appendix A)

3.7 Inclusion Criteria

- Adult male or female (>18 years old)
- Positive Serology for Hepatitis B Surface antigen (HBsAg) at diagnosis
- Detectable Serum HBV DNA by quantitative PCR assay prior to lamivudine initiation
- Never received prior antiviral therapy for HBV infection before

3.8 Exclusion Criteria

- Co-infection with Hepatitis C Virus or HIV
- Patient receiving steroids or immunosuppression (such as chemotherapy)
- Patient with organ transplant
- Known to be pregnant or breastfeeding

## 3.9 Definitions of outcome measurement used in this study

### 3.9.1 Lamivudine Resistance to HBV

In this study, we defined lamivudine resistance as patient who is currently or previously on lamivudine given as first line monotherapy for HBV infection and developed **any** of these 3 following criteria while on lamivudine therapy:-

(A) Virological breakthrough - Increase of HBV DNA > 1 log IU/ml from nadir

(B) Biochemical breakthrough – increase of ALT by 2 times of nadir

(C) Increase of HBV DNA and/or ALT level that are not up to the level to meet Criteria (A) or (B), nonetheless there is documentation stated that the treating gastroenterologist decided to discontinue lamivudine and change to other anti-viral against HBV in the absence of other factor such as intolerable drug side effects.

This definition is rather very liberal definition for lamivudine resistance. Previous studies that look at the resistant rate used virological breakthrough or genotypic detection, which in our setting are not routinely done. Biochemical breakthrough as a surrogate marker lamivudine resistance has been evaluated in one study by Jang et al (Jang *et al.*, 2005). The liberal use definitions of resistance in this study will have more impact on the clinical practice if we are able to demonstrate lower resistance rate compared to historical rate.

Resistance to lamivudine was reported in terms of its period prevalence and cumulative incidence. In this study, period prevalence of lamivudine resistance is the number of patient who developed resistance to lamivudine divided by total number of patient who is on lamivudine over a 6 years period of 2010 to 2016.

Cumulative incidence of lamivudine resistance is calculated by following formula

$$\text{Cumulative Incidence} = \frac{\text{Number of new case of lamivudine resistance}}{\text{Number of subjects on lamivudine at risk}}$$

### 3.9.2 Treatment Response

Treatment response after lamivudine initiation was assessed via several parameters. Subjects were regarded as having response to lamivudine if meet any one of these 3 criterias:

1. Virological Response – HBV DNA load of <2000IU/ml after 6 months of starting lamivudine
2. Biochemical response – normalization of ALT level
3. Serological response – HBeAg loss in a previously HBeAg positive patient

### 3.9.3 Variable Definition

Duration of treatment

Calculated in months from date of lamivudine initiated to the date it was discontinued. It may be discontinued due to following reasons: Development of resistance or because of total treatment duration have deemed completed by treating gastroentroentrologist.

Pre-treatment HBV DNA load

Measured HBV DNA viral load in IU/ml before the initiation of lamivudine therapy

Pre-treatment ALT level

The last measured serum alanine transaminase before the intiation of lamivudine therapy. In addition to the absolute value (in IU/L), ALT level also was categorized into 3 groups – normal, minimally raised and raised. Normal ALT is defined as ALT level within labarotory reference range. Minimally raised serum ALT level is defined as increment of ALT level up to two times upper limit normal, and raised serum ALT is define as increment of more than two times upper limit normal.

Baseline HBeAg status

Presence of HBe antigen before the initiation of lamivudine therapy

Cirrhotic status

Presence or absence of liver cirrhosis based on the result of the ultrasound abdomen done at any point during the course of follow up

## 3.10 Sample Size Calculation

**Objective 1**

Sample size is based on overall prevalence of lamivudine resistance of 35% (Osman, 2011)

$N = [z / \Delta]^2 p ( 1 – p )$

P = prevalence of lamivudine resistance 0.35

Z = 1.96 for 95% CI

Δ (precision) = 0.1

$N = [ 1.96 / 0.1]^2 0.35 ( 1 – 0.35 )$

= 87

Missing data estimated to be 10%

**The sample of 96 will be required at analysis stage.**