TREATMENT OUTCOME OF CHRONIC HEPATITIS B GENOTYPE B AND C AT HOSPITAL FOR TROPICAL DISEASES, BANGKOK, THAILAND

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on
April 29, 2010

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ABSTRACT

Currently, two classes of drugs are used for the treatment of chronic hepatitis B (CHB), interferon and nucleos(t)ide analogue (NA). NAs are widely used in Asia including Thailand. There are eight different genotypes of hepatitis B in different regions of the world. In Thailand, majority is genotype C and minority is genotype B. Genotype C infection has more severe disease and higher risk of hepatocellular carcinoma. However, genotype-specific treatment outcome is inconsistent in different ethnicity. Whether treatment outcome of genotype B and C differ after NA therapy is unknown among Thai patients.

The objective of the study was to compare the treatment outcome of CHB genotype B and C patients after sixth months of NA therapy. Primary outcome was undetectable hepatitis B virus DNA (HBV-DNA) less than 3 $\log_{10}$ copies per milliliter.

Forty CHB patients attending to the liver clinic of Hospital for Tropical diseases, Bangkok, from 2004 to 2009 were studied in retrospective cohort design. Six genotype B patients (15%) and thirty-four genotype C patients (85%) were treated. All were treatment naïve patients and Thai ethnicity. Serum HBV-DNA level, serum alanine amino transferase (ALT) level, HBeAg status and alpha-feto protein (AFP) level were measured at the start of NA therapy and again at six months of treatment.

Baseline data of patients in genotype B and C were comparable. After six months of NA reatment, achievement of undetectable HBV DNA was higher in genotype B patients (66.7%) compared to genotype C patients (42.4%) [RR=1.57, 0.79-3.14] (p= 0.387). ALT normalization was more common in genotype B (50%) than in genotype C (29.7 %) (p= 0.381). HBeAg conversion was observed in (10 %) of genotype C cases but not in genotype B cases. Median AFP level of two genotype groups were not different significantly (p= 0.317).

Despite the more severe natural course of genotype C in existing evidence, CHB genotype B and C were not significantly different in term of treatment outcome after six months of NA therapy.

KEY WORDS: CHRONIC HEPATITIS B, GENOTYPE, TREATMENT, THAILAND

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LIST OF ABBREVIATION

Abbreviation or symbol

AASLD	American Association for the Study of Liver
AFP	alpha-feto protein
ALT	alanine aminotransferase
anti-HBe	antibody to hepatitis B e antigen
anti-HBs	antibody to hepatitis B surface antigen
anti-HCV	antibody to hepatitis C virus
anti-HIV	antibody to human immunodeficiency virus
APASL	Asian Pacific Association for the Study of the Liver
AST	asparatate aminotransferase
CHB	chronic hepatitis B
EASL	European Association for the Study of the Liver
HBV	hepatitis B virus
HBV-DNA	hepatitis B virus DNA
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HCC	heaptocellular carcinoma
HIV	human immunodeficiency virus
Log	logarithm (to base 10: common logarithm)
NA	nucleos(t)ide analogue
NAs	nucleos(t)ide analogues
n	sample size
N	sample size
ml	milliliter
ng	nanogram (10^{-9})

LIST OF ABBREVIATION (cont.)

P or *p*	P-value (probability of getting a result statistic as extreme as or more extreme than the one observed)
PCR	polymerase chain reaction
UNL	upper normal limit
US panel	Panel of United States hepatologists or Keeffe's guideline
<	Less than
≤	Less than or equal to
>	greater than
≥	greater than or equal to
/	per
%	percent

CHAPTER I
INTRODUCTION

1.1 Background and significance

Hepatitis B is a disease of global burden. Prevalence of hepatitis B virus (HBV) is extremely high. Hepatitis B virus infects one third of the world population. There are more than 350 million cases of chronic hepatitis B and worldwide (1). These people have different clinical status ranging from asymptomatic infection to severe liver disease. They have very high risk for progression to hepatocellular carcinoma (HCC) with relative risk 9.6 for HBsAg positive and relative risk 60.2 for both HBeAg and HBsAg positive patient (2).

Existing evidence showed that hepatitis B virus DNA (HBV-DNA) is important for the carcinogenesis. Large cohort studies showed higher serum HBV-DNA level is associated with increasing risk of HCC in chronic hepatitis B patients (3, 4). Infection by HBV genotype C was reported to be strongly associated with the development of HCC, adjusted relative risk of 10.24 (5).

Currently, eight genotypes of hepatitis B virus have been recognized, genotype A to H (6). Different genotypes of HBV are prevalent in various regions of the world. Genotype A and D are major HBV genotypes types in Europe and America. In south East Asia genotype, B and C are the major types (7-9). According to previous nationwide survey result, genotype C and genotype B are two major predominant strains in Thailand (10).

The goal of treatment in chronic hepatitis B is to reduce the risk of HCC and severe liver disease by lowering HBV replication and limiting the progressive liver damage (11-14). Currently there are two kinds of treatment for chronic hepatitis B namely interferon therapy and nucleos(t)ide analogue (NA) therapy (15). There is strong evidence that both of these antiviral therapies can significantly reduce the risk

of HCC (16). Four NAs such as lamivudine, telbivudine, entecavir, and adefovir have been approved and widely used in treatment of chronic hepatitis B in Asia (15).

Different clinical nature and different risk of HCC between infection by HBV genotype B and C was found in Asian population (5). Studies in Thailand also reported the different features in genotype B and C (17). Moreover, it was reported that Genotype B had better response to interferon than genotype C by different studies of different region (18-20). However, response of genotype B and C to widely used NA therapy was reported by very few studies in Asian patients and the result were not conclusive (21-23). The nature of response to NA by major genotypes B and C in treatment naïve population is yet to be established in Thailand. This study is aimed to find out the treatment outcome of chronic hepatitis B genotype B and genotype C to NA therapy.

1.2 Primary objective

1. To compare the proportion of patients achieved undetectable HBV DNA level at sixth month after nucleoside analogue treatment between chronic hepatitis B genotype B and C groups.

1.3 Secondary objectives

1. To compare the proportion of patients achieved ALT normalization between genotype B and C at sixth month after nucleoside analogue treatment

2. To compare the proportion of patients achieved HBeAg seroconverison between genotype B and C at sixth month after nucleoside analogue treatment

3. To compare the changes in AFP level between Genotype B and C at sixth month after nucleoside analogue treatment

1.4 Operational definition

Undetectable HBV DNA level means HBV DNA level that is not detectable as it is less the minimum detectable level of the technique used for individual case. It may be different from case to case. The level of reduction will be assessed based on the comparison of the serial result by using single technique. As a matter of generalization in this study, undetectable viral load means HBV DNA less than 3 $\log_{10}$ copies per ml.

HBeAg seroconverison means disappearance of HBeAg and appearance of anti-HBeAg antibody in patient's serum.

ALT normalization means ALT level less than 30 IU/ml in the male and less than 19 IU/ml in the female (14).

CHAPTER II
LITERATURE REVIEW

2.1 Global burden of the disease

Hepatitis B is significant cause of worldwide morbidity and mortality. According to World health organization report 2006 one third of the world population is infected (1). There are 4 million acute cases per year and 1 million deaths per year. Chronic hepatitis B is one of the most common chronic infections to humankind. In addition, there are 350 – 400 million cases of chronic hepatitis B cases. Being the major cause of hepatocellular carcinoma (HCC), it is included in the list of world top ten leading causes of death (1). It is the second most common cause of carcinogen behind tobacco. Sixty to eighty percent of all the primary liver cancers are associated with hepatitis B.

2.1.1. HBsAg prevalence

HBsAg seroprevalence is reflecting the endemicity of hepatitis B. It is ranging from less than 0.5 % in United States to more than 8 % in Asian Pacific countries differently all over the world.

Hepatitis B is endemic in Taiwan, China and other parts of Asia. Thailand is also one of the endemic areas. Most of the patients in the region become infected with HBV during childhood. In these regions, 8% to 10% of the adult populations are chronically infected (1). Liver cancer caused by HBV is among the first three causes of death from cancer in men, and a major cause of cancer in women.

Table 2.1 HBsAg seroprevalence (24)

Country	***HBsAg positive percentage***
Taiwan	10.0 -13.8
Vietnam	5.7-10.0
China	5.3-12.0
Africa	5.0-19.0
Philippine	5.0-16.0
Thailand	4.6-8.0
Hong Kong	4.5-12
Japan	4.4-13.0
Indonesia	4.0
South Korea	2.6-5.1
India	2.4-4.7
Russia	1.4-8.0
Canada	0.5-1
United States	0.2-0.5

2.1.2. Vaccination and change of prevalence

Hepatitis B vaccine was started in universal immunization program in 1991. After introduction of vaccination program, incidence of hepatitis B declined 80% in United States. Nevertheless, the disease prevalence is still high in many areas of the world.

2.1.3 Mode of transmission

Hepatitis B virus can be transmitted horizontally as well as vertically. The routes of transmission can be per mucosal as well as per cutaneous. Not only blood and serum, all the body secretion of the infected person have infective potential. The significant amount of HBV virus DNA is found in saliva, sweat and urine of the chronic hepatitis B patients (25). Mode of transmission is the same for the human immunodeficiency virus (HIV). However, HBV is 50 to 100 times more infectious than HIV. The infective dose is very small, 0.001 ml. Moreover, HBV can survive outside the body for at least 7 days.

In the endemic area, vertical transmission to the baby from mother is common. Infection during infancy, unsafe injection and transfusion of infected blood is also common in developing countries (1). In the area of low endemicity like United States and western European countries, patterns of transmission are different from Asia-Pacific endemic area Sexual transmission and transmission through injecting drug use is common among young adult. HBV is a major infectious occupational hazard of health care professionals (1).

HBV is not spread by contaminated food or water, and cannot be spread casually in the workplace. The virus incubation period is 90 days on average, but can vary from about 30 to 180 days. HBV may be detected 30 to 60 days after infection and persist for widely variable periods (1).

2.1.4 Risk factors

The following populations are at the risk factors for hepatitis B virus infection (1).

- Infants of HBV infected mother
- Children with infected family members
- Sexual partner of the infected persons
- Non monogamous sex
- Injection drug users
- Men who have sex men
- Households contact of hepatitis B patient
- Blood transfusion without proper screening policy
- Health care workers especially those who are handling the blood and blood products
- Haemodialysis

2.1.5 Epidemiological and clinical correlation

Clinical pattern of the disease and nature of the disease is related to epidemiological pattern of the disease. In endemic area like Asia-pacific countries, infection at birth and at early age is common. The risk of getting infection from infected mother is very high especially if the mother is HBeAg positive (26). The risk of becoming chronic hepatitis which can progress to end stage liver disease and HCC is 90% if infected at birth and 40% if infection in infancy (27, 28).

In contrast, in low endemic area like United States, infection at young adult is common. Ninety percent of adulthood infections recover and get rid of virus completely (1). Chronic hepatitis is not frequent, so the risk of end stage liver disease and HCC is low.

2.2 Natural history of hepatitis B

Hepatitis B virus is enveloped double-stranded DNA virus. HBV replicates its genome via RNA intermediate. The main cellular target for HBV is hepatocyte. After the entrance to hepatocyte, the viral genome is integrated into host genome in the form of covalently closed circular DNA (cccDNA) in hepatocye nuclei (29). Replication of HBV has a distinct step, reverse transcription of RNA intermediate that is transcribed from cccDNA to form viral DNA. The property is supposed to give the virus high rate of mutation (29).

Subsequent to being infected by HBV, usually immune-competent adults recover after acute infection, which is mostly asymptomatic. About 95 % of the immune-competent adults can have immune response that can clear the infection within a few weeks. About 5 % supposed to have impaired HBV specific immune response cannot clear the infection and acquire chronic hepatitis after primary infection (30).

Understanding of natural history of chronic hepatitis B infection has been progressively increased in recent two decades. Accessibility of more sensitive DNA assays and better understanding of host immune response to HBV has changed the concept of the natural history of chronic hepatitis B.

2.2.1 Old concept and current concept of chronic hepatitis B

Throughout the course of chronic hepatitis B, there is persistent viral replication. Liver injury is because of dynamic interaction between host immune response and viral replication. In old concept, natural history had two phases: initial phase with presence of HBeAg, HBV DNA and active liver disease and late phase characterized by absence of HBeAg, undetectable serum HBV DNA and inactive liver disease.

In the current concept, natural history of chronic hepatitis B is recognized as four phases although not all patients go through these phases (28).

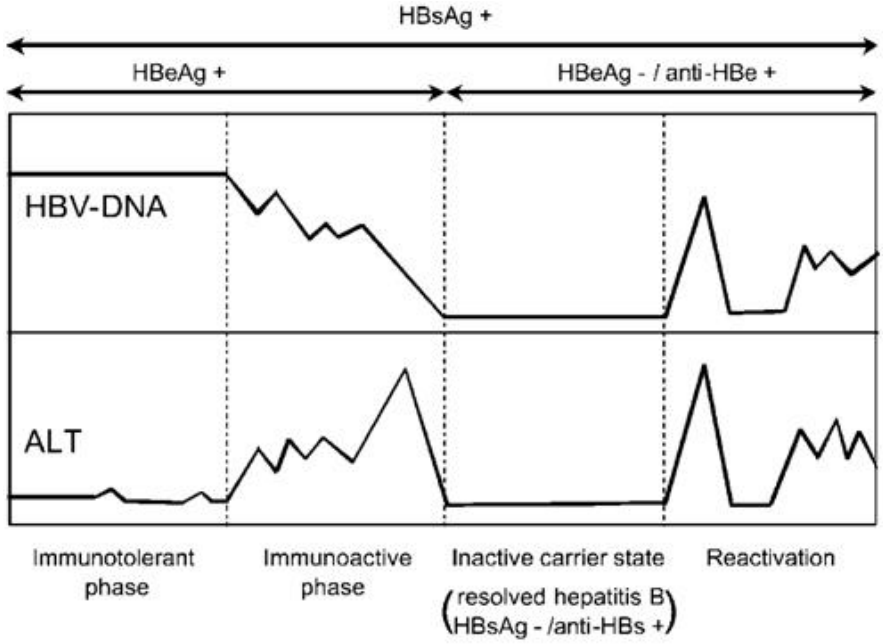


Figure 2.1 Four phases of chronic hepatitis B natural history (31)

2.2.2 Phases of the natural history of chronic hepatitis B

1. Immunotolerant phase
2. Immunoclearance or immunoactive phase
3. Inactive HBsAg carrier or low replication phase
4. Reactivation phase

2.2.2.1 Immunotolerant phase

The characteristic features of patients in this phases are positive HBeAg, high HBV-DNA, normal transaminase, and minimal or no inflammation on liver biopsy. Infection acquired at birth or during infancy usually have long duration.

2.2.2.2 Immuno-clearance phase /immune-active phase

The characteristic features are positive HBeAg, high or fluctuating serum HBV-DNA level, intermittent or persistent increase in transaminase, and active inflammation on liver biopsy. Another distinct feature in this phase is flare up of transaminase. Duration of immune-clearance phase and frequency of flare up are

directly associated with the risk of cirrhosis and hepatocellular carcinoma (32, 33). HBeAg loss and conversion to antiHBe antibody can occur after this phase.

2.2.2.3 Inactive HBsAg carrier state

The characters of patients in this phase are HBeAg negativity, presence of antiHBe antibody, persistently normal transaminase level, low or undetectable viral DNA, and mild hepatitis and minimal fibrosis. The inactive carrier state can last a considerably long duration that suggests the favorable outcome especially if the patient gets carrier state very early (34). In contrary, 1.46% spontaneous relapse per year (20% on 25 years follow up) was found in a Taiwanese study of 1241 carriers on 25 years follow up (35).

2.2.2.4 Reactivation phase

This phase is synonymous with HBeAg negative chronic hepatitis. It is thought to be consequence of reactivation of the viral replication. Patients in this phase are characterized by HBeAg negativity, presence of anti-HBe antibody, detectable HBV DNA, elevated aminotransferases and ongoing necro-inflammation in liver histology. The risk of HBeAg negative chronic hepatitis B to cirrhosis and HCC is described differently by studies from Asia and Mediterranean and European large cohort studies.

2.2.3 Clinical spectrum

The annual risk of developing cirrhosis and HCC of all above four phases are summarized in figure 2.2.

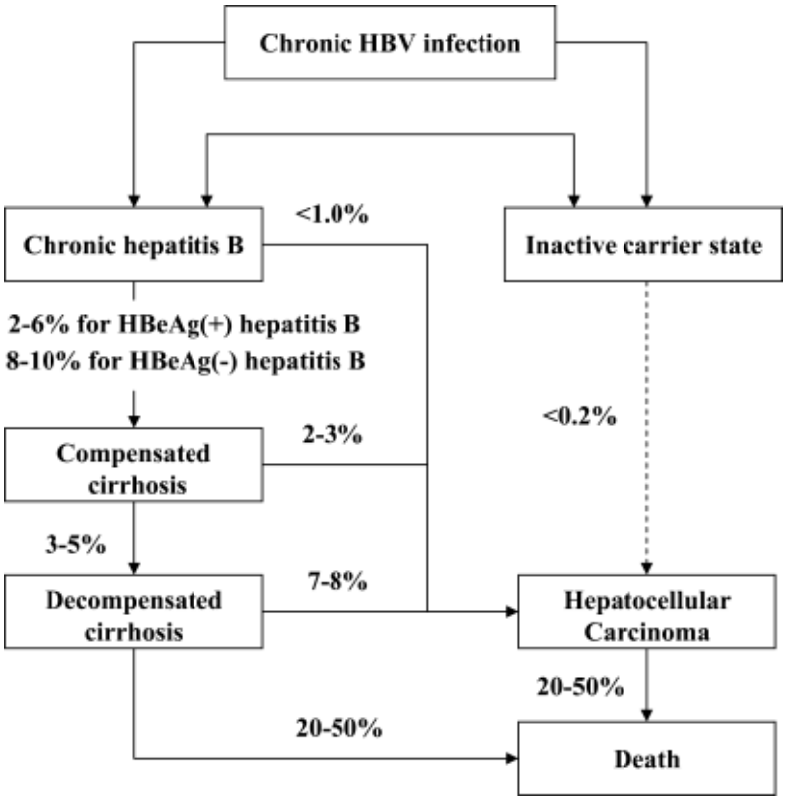


Figure 2.2 Estimated annual rate percentage of progression through different clinical stages during the course of chronic hepatitis B infection (28)

Clinical spectrum of chronic hepatitis B can be HBeAg positive chronic hepatitis B, HBeAg negative chronic hepatitis B, Inactive HBsAg carrier state, and long-term complication of chronic hepatitis B like cirrhosis of the liver, hepatocellular carcinoma. Spontaneous clearance of HBsAg was reported and at the rate of 0.5% to 1% per year in chronic hepatitis patients (35, 36). However, this rate is lower in endemic areas.

Studies in endemic region like in Asia like China and Taiwan and Japan had shown higher relative risk for HCC than in Western countries. It was correlated to mode of transmission as vertical transmission and infection in infancy is common in endemic.

HBeAg negative hepatitis B was common in Mediterranean countries like Greek but nowadays it is frequent globally. Scientific evidence confirmed that HBeAg negativity is because of mutant virus.

2.3 Hepatitis B genotypes

2.3.1 Global distribution of HBV genotypes

Currently there are eight known genotypes of hepatitis B namely A to H all over the world. Global distribution of hepatitis B genotypes are already described well by Miyakawa and Mizokimi in 1988 (6). Genotype B and C is confined to Asia while in US and European countries and in most of the Africa, A and D genotypes are prevalent (6). In India, genotypes A and D are prevalent (37). Genotype G cannot be specified to any particular region. Genotype F is common in Alaska (38) and Genotype H is seen in Amerindians (9). Major genotypes in Thailand are genotype B and C (10).

Table 2.2 Genotypes of hepatitis B in different countries (39)

Genotype	Geographical region
A	Northwestern Europe, Spain, Poland, USA, Central Africa, India, Brazil
B	Southeast Asia, Taiwan, Japan, Indonesia, China, Hong Kong, Vietnam, Thailand
C	Far East Asia, Taiwan, Japan, Korea, China, Hong Kong, Thailand, Indonesia, Polynesia, Solomon Islands, Vietnam, India, Australia, USA, Brazil
D	Mediterranean area, Albania, Middle East, Turkey, Iran, India, Spain, Czech, Russia, USA, Brazil, Solomon Islands
E	West Africa
F	Central and South America, Bolivia, Venezuela, Argentina, Brazil, Polynesia, Alaska
G	France, Germany, USA
H	Central and South America

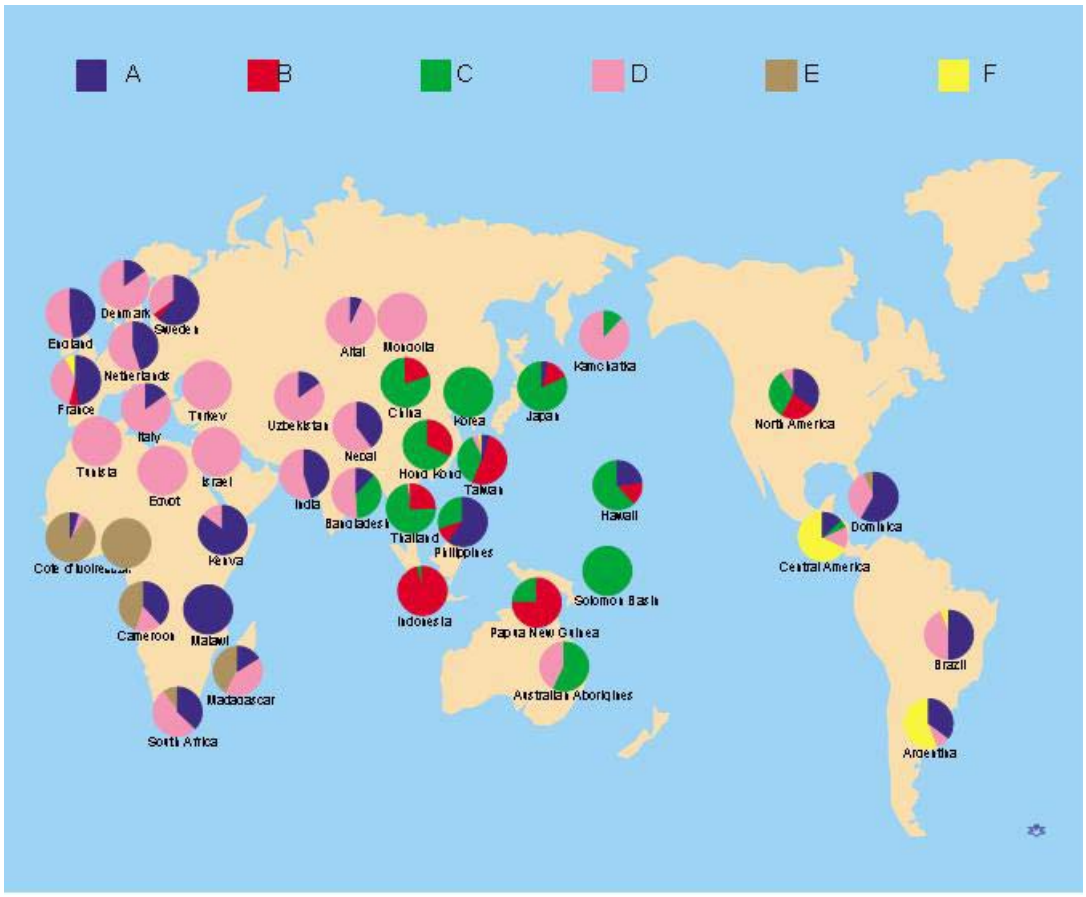


Figure 2.3 Distribution of the major six HBV genotypes worldwide (6)

2.3.2 Significance of hepatitis B genotypes

Hepatitis B genotypes become increasingly interested in recent years. Other than the geographical diversity, evidences showed that difference in clinical pattern of chronic hepatitis B is not because of race but because of different genotype of HBV (40). One study reported that survival among HCC of two genotypes is not different. HBV genotypes might have therapeutic implication as different genotypes can cause distinguished clinical presentation and clinical outcome, and different response to antiviral therapy.

2.3.4 Conclusive and contradictory results of previous studies; Genotype B vs. C

Table 2.3 Comparison between hepatitis B genotype B and C

	Genotype B	**Genotype C**
Clinical		
Cirrhosis and HCC incidence	Lower	Higher
Relative Risk of HCC (41, 42)	Lower risk (RR=0.3)	Higher (RR=2.6)
Age of HCC patients	< 35, younger	Older
Liver disease association	Non-cirrhotic	Severe liver disease
Over all outcome(38)	Better	Worse
Acute exacerbation	Not different/ Not known	
Histological	Lower	Higher
Liver biochemistry	Not different / Not known	
Virological		
HBeAg positivity	Less	More
HBeAg seroconversion (40, 42)	Earlier, higher	Later, lower
Flare after HBeAg loss	Less	More
Immunoclearance phase	Shorter	Longer
HBsAg clearance	More	Less
Spontaneous HBeAg seroconversion	Higher	Lower
Pre-core stop codon mutation	More	Less
Basal core promoter mutation	Less	More
Serum HBV-DNA level	Lower	Higher
Break through	Not known	
Response to anti viral		
Standard interferon (18-20)	Higher	Lower
Peg interferon ((20)	Higher	Lower
Lamivudine (21-23)	Higher	Lower
Drug resistance mutation	Controversial	
Adefovir	Comparable	
Entecavir	Not known	
Telbivudine	Not known	

Large cohort studies in Asia had shown evidence that genotype C is a stronger risk factor for HCC than genotype B (5). Moreover, Asian studies consistently found that genotype C is definite risk factor for development of HCC and type C has higher risk of HCC than any other types in their region. It has severe disease progression than genotype B (5, 41, 43).

Taiwanese study and Chinese studies had the same finding of genotype C as independent risk factor for HCC (5, 39). Another agreed finding is association of genotype B to better outcome of the disease. However, in case of genotype B patients with HCC, younger age is a consistent finding. According to the result of study in Chinese population and Taiwanese population HBeAg, seroconversion rate is much different. It may be because of difference in follow up period. Eventually clinical pattern and outcome related to genotypes of hepatitis B still have contradictory result even from the same region, Asia.

2.3.5 Clinical significance of genotype C

In CHB patients with genotype C, HBeAg conversion is reported to be slower than those infected with genotype B. Incidence of HCC is higher with adjusted relative risk 10.42 in genotype C (5). Therefore, genotype C has been recognized as a poor prognostic factor. In a study of Japanese patients, the rate of progression to HCC was faster and higher in genotype C patients of age group 30 and older (44). Therefore, genotype C is strong predictor of HCC. The disease course and severity is more severe in genotype C**.**

2.3.6 Genotypes of hepatitis B in Thailand

Epidemiological studies reported that the major genotypes in Thailand are genotype C and genotype B (17, 45, 46). The prevalence of genotype C and B in Thailand is 73% and 21% in a hospital-based study and, 87.1% and 11.6% in population-based survey (10, 46). Genotype C is more prevalent than genotype C in Thailand.

2.3.7 Conclusive and contradictory results of previous studies; Genotype A vs. D

Between the genotype A and D, which are common in European, US and India, western studies had pointed out the better outcome of genotype A than genotype

D (39). However, study in India reported the result of no significant difference between genotype A and D (37). Regarding the response to antiviral there is no conclusive finding apart from different response to interferon therapy by genotype A vs. D and B vs. C.

Table 2.4 Comparison between hepatitis B genotype A and D (39, 43)

	Genotype A	**Genotype D**
Clinical course		
Cirrhosis and HCC incidence	Lower	Higher
Risk of HCC	Lower	Higher
Liver disease association	Less severe	More severe
Overall outcome	Better	Worse
Acute exacerbation	Less	More frequent
Histological	Better	Worse
Acute hepatitis	Less common	More common
Virological		
HBeAg positivity	Evidence not conclusive yet	
HBeAg seroconversion	Higher	Lower
Sustained viral response	Higher	Lower
Response to drugs		
Standard interferon	Better	lower
Peg-interferon	Better	lower
Lamivudine	Better	lower
Drug resistance	Higher	lower
Adefovir	Not known well	
Entecavir	Not known yet	
Telbivudine	Not known yet	

2.4 Current treatment of hepatitis B

2.4.1 Aim of the therapy

Current goal in treatment of chronic hepatitis B is to reduce the HBV viral replication as low as possible in a sustained manner to reduce the risk of hepatocellular

carcinoma and progression to severe liver disease and improve the quality of life.

Therefore reduction of HBV DNA level as low as possible to an undetectable level is the primary aim of the therapy. Achieving this aim in turn gives rise to other aims of the therapy such as normalization of liver enzymes, histological improvement, HBeAg seroconverison in case of HBeAg positive chronic hepatitis B, and HBsAg seroconverison in very few cases (11-14). Long-term goals are to reduce development of cirrhosis and hepatocellular carcinoma and ultimately to extend the survival.

2.4.2 Drugs

Two classes of drugs are currently used for treatment of chronic hepatitis B. These are interferon therapy and NA therapy. Seven drugs have been approved. Drugs currently used in the interferon class are Interferon alfa-2b, peg interferon alfa-2a. NAs are lamivudine, adefovir, telbivudine, entecavir, and tenofovir.

2.4.3 Indication for the treatment

Table 2.5 Recommendation for treatment in CHB patients in guidelines

	HBeAg positive		**HBeAg negative**	
Guidelines	HBV DNA copies /ml	ALT	HBV DNA copies /ml	ALT
EASL (13)	$\geq 10^5$	>2×ULN*	$\geq 10^5$	>2×ULN
US panel (14)	$\geq 10^5$	>ULN	$\geq 10^4$	>ULN
Asia-pacific panel (11)	$\geq 10^5$	>2×ULN	$\geq 10^4$	>2×ULN
AASLD (12)	$\geq 10^5$	>2×ULN	$\geq 10^5$	>2×ULN

*ULN= upper limit of the normal

Table 2.6 Recommendation for treatment in CHB patients with cirrhosis

	HBeAg positive		**HBeAg negative**	
Guidelines	HBVDNA copies/ml	ALT	HBV-DNA copies /ml	ALT
EASL (13)	$\geq 10^5$	Abnormal	$\geq 10^5$	Abnormal
US panel (14)	$\geq 10^4$	No ALT specified	$\geq 10^4$	No ALT specified
Asia-pacific panel (11)	$\geq 10^4$	No ALT specified	$\geq 10^4$	No ALT specified
AASLD (12)	$\geq 10^5$	No ALT specified	$\geq 10^4$	No ALT specified

The advantages and disadvantages of these two classes of drugs are compared as followed in EASL guideline (13)**.**

Table 2.7 Peg interferon and nucleos(t)ide analogue

	Pegylated interferon alpha	**Nucleos(t)ide analogue**
Advantages	Finite duration Absence of resistance Higher rate of HBeAg and HBsAg conversion	Potential antiviral effect Good tolerance Oral administration
Disadvantages	Moderate antiviral effect Poor tolerance Subcutaneous injection	Indefinite duration Risk of resistance Lower rate of HBeAg and HBsAg seroconversion

2.4.4 Efficacy of the drugs

The following table shows efficacy of currently used drugs compared in term of virological response at one year in EASL guideline.

Table 2.8 Comparison of drugs in treatment of CHB by means of virological response at one year (12, 13)

	Virological response (%)at one year by undetectable HBV-DNA*		**HBsAg loss (%)**
	HBeAg +	HBeAg -	
Peg-Interferon alpha 2a	25	63	3-4
Lamivudine	36-40	72	0
Adefovir	21	51	0
Telbivudine	67	90	0
Entecavir	60	88	0
Tenofovir	74	91	0

*The definition of undetectable HBV-DNA is not the same in different trial and different from the currently accepted guidelines.

2.5 Parameters used for the assessment of treatment outcome

In the treatment of chronic Hepatitis B, current therapy cannot completely clear the virus so far. Nevertheless, outcome and effectiveness of the treatment can be known by following parameters.

2.5.1 HBV-DNA level

HBV-DNA level reflects the viral replication. The risk of progression to cirrhosis is strongly associated with the level of circulating virus (4). There are strong evidences that showed the association of HBV-DNA level and risk of hepatocellular carcinoma (3, 47, 48). With the higher the viral load, the risk for development of cirrhosis, its complication and HCC becomes greater. It is useful for monitoring of the disease process (49).

Because of more sensitive, improved molecular technology, HBV-DNA level can be detected up to 10 -15 copies /ml by real-time PCR essay. However, minimum undetectable level may differ in different centre depending on the technique used.

After receiving NA therapy, the response to the treatment is determined by achievement of undetectable level of HBV-DNA by real time PCR. EASL guideline suggests the end- point of the therapy as HBV-DNA level as low as undetectable by real-time PCR essay (10 to 15 IU/ml) to ensure the virological suppression that may lead to normalization of liver enzymes, histological improvement and prevention of complication (13).

2.5.2 HBeAg seroconverison

HBeAg seroconversion means loss of HBeAg and appearance of anti-HBe antibody. It is a good indicator of response in HBeAg positive chronic hepatitis B. HBeAg seroconversion was seen in 30% with interferon therapy and 20 % with nucleoside analogues. Nevertheless, the presence of another class of disease HBeAg negative hepatitis is a major limitation.

2.5.3 HBsAg seroconversion

HBsAg is very useful for screening. Persistence of HBsAg more than six months indicates that infected person has acquired the chronic infection. HBsAg seroconversion means disappearance of HBsAg and appearance of anti HBs antibody. It is an ideal indicator of the cure of disease. However, loss of HBsAg after one year of therapy is only about 1 to 3% (12, 13). Therefore, it is not realistic to monitor the treatment response by HBsAg seroconversion. Currently quantitative assessment of HBsAg becomes interested. However, the HBsAg titer between genotype B and C is found to be not different (50).

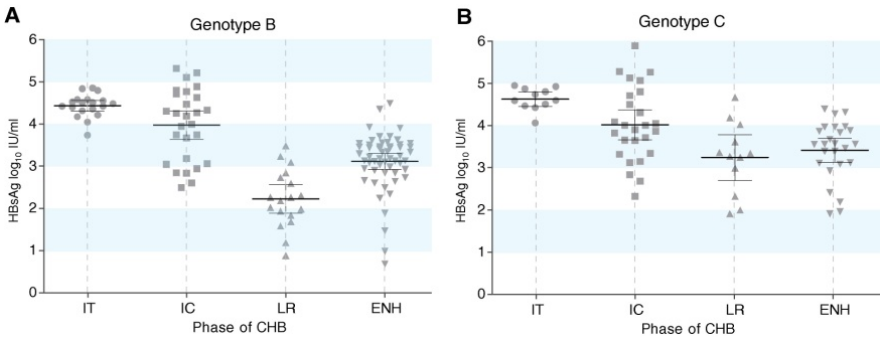


Figure 2.4 Distribution of serum HBsAg titers in (A) CHB genotype B, and (B) CHB genotype C: Median values with 95% confidence interval represented (50)

2.5.4 Serum aminotransferase ALT and AST

The biochemical markers are reflecting the severity of the inflammation in liver and injury to hepatocytes. However, ALT is sensitive to detect the advanced liver disease and cirrhosis without symptom. Even slightly increased in ALT level above normal is associated with increase in long-term mortality (51). Evidence pointed out ALT level as prognostic markers in hepatitis B in Asian population (52).

ALT level is considered in context with HBV DNA level to decide the indication for the treatment in non-cirrhotic chronic hepatitis B patients. As an endpoint of therapy, these markers do not have the individual value because of their fluctuating nature throughout the course of the disease. Recently, lower normal value of ALT was suggested by the guide-lines to be considered at normal value for male at 30 IU/L and female at 19 IU/L lower level differently for male and female (14).

2.5.5 Liver biopsy

Liver biopsy and histological examination is the accurate assessment of liver injury. It can rule out the other causes of liver diseases. However, it is invasive and costly. Liver biopsy is useful in persons who do not meet the clear-cut indication for the treatment in guidelines as described above.

2.6 Screening of cancer

Alpha-fetoprotein (AFP) and ultrasound are routinely used for early detection of HCC. Increased AFP level above 20 ng/ml alone is an independent prognostic factor for disease progression to decompensated cirrhosis with the relative risk of 3.75 (53). Screening of HCC by using AFP and ultrasound together has detection rate of 92% with false positive rate 7.5% (54). HCC can be detected at an earlier, surgically resectable stage. Screening and early detection has significant benefit on longer survival rate in chronic hepatitis B patients who developed HCC (55).

2.7 Effect of the treatment

Recently a meta-analysis of randomized trials, case control and cohort studies over last ten years showed that antiviral therapy can prevent the liver cancer. The risk of HCC is reduced by 34% after treatment with interferon (relative risk for

HCC=0.66), 78% after nucleoside analogue therapy (relative risk for HCC=0.22) (16). The result was concluded as interferon has more benefit in cirrhotic patients and nucleoside analogues had more benefit on non-cirrhotic and HBeAg positive chronic hepatitis patients.

2.8 Influence of genotypes on drug response

The response to antiviral therapy in HBV depends on host and viral factors. Genotypes of HBV become interested as an influence on response to antiviral therapy. It is well established that HBV genotype has influence on progression of the disease and the risk of HCC. Nevertheless, drug response in the scope of genotype has not been specified well.

On reviewing the four current guidelines, recommendation for genotyping is different.

Table 2.9 Recommendation for genotyping in current guidelines

	Year of publication	**Recommendation for genotyping**	**Impression**
AASLD (12)	2007	Optional	Important factor for response to antiviral
EASL (13)	2009	Optional	Predictor of response in interferon therapy
Asia-Pacific (11)	2008	Optional	Important factor for response to antiviral
US panel (14)	2008	pre-treatment evaluation test	Routinely/selectively

Among guidelines, US panel is the only consensus recommending genotyping in the patients with chronic HBV infection selectively to identify the patients at risk of more severe progressive liver disease, and routinely with the use of peg-interferon therapy (14). AASLD guideline described the HBV genotyping as predictor of response for HBeAg seroconverison in HBeAg positive patients receiving interferon therapy and highlighted the need of additional data for role of genotype in nucleoside analogue therapy. However, EASL guideline described that genotype alone

does not have individual predictive factor for interferon therapy and does not influence the response to any NA as evidences were not strong enough yet (13).

2.9 Drug response between genotype B and C

2.9.1 Interferon

Response to interferon therapy is different among genotype B and C. Genotype B responds better to interferon therapy than genotype C according to existing evidence. Study in Taiwanese population reported in term of response rate defined as normalization of ALT, HBeAg loss and HBV DNA 48 weeks after treatment. The response to interferon is reported as (41%) in genotype B versus (15%) in genotype C, (P=0.045) (19). In Chinese population antiviral response after interferon–alpha treated patients was 39% in genotype B versus 17 % in genotype C (P=0.03) respectively (18). Post hoc analysis of a randomized controlled trail in China also showed the similar result (20).

2.9.2 Nucleos(t)ide analogues

NAs are widely used because of their safety profile as well as efficacy. Among the nucleoside analogue currently used for treatment of chronic hepatitis, available data are stating that influence of genotype on NA therapy is not yet conclusive.

2.9.2.1 Lamivudine

Response to lamivudine was reported differently in different geographical region in Asia by previous studies. Study in Hong Kong reported outcome of lamivudine 100 mg daily with response rate defined as HBeAg seroconverison, normalization of ALT, and sustained response six months after treatment (21). The response to lamivudine was reported as (14%) in genotype B versus (10%) in genotype C, (P=0.41). However, the sample size of that study was only 35 patients on lamivudine and 96 patients as control. The difference was not significant. The same insignificant result was reported by a Taiwanese study. This non-significant result might also be due to small sample size (n=31) (56).

Study in Taiwanese population reported the sustained response after lamivudine therapy in term of response defined as normalization of ALT, loss of serum HBV-DNA and seroconverison of HBeAg to anti- HBe antibody. The result

was better outcome in genotype B patients 61% versus 20 % in genotype C patients (n=82, P=0.09). Genotype B infection have much higher sustained response than those with genotype C infection (23).

Japanese study described the role of genotype in predicting long-term outcome of lamivudine and emergence of drug resistant mutation comparing genotype A, B and C (22). Notably the clearance of HBV DNA from serum of the patients on long-term lamivudine more than 192 weeks was 84% in genotype B and 76% in genotype C.

2.9.2.2 Adefovir

The response to adefovir dipivoxil is also expected to be different in genotype B and C. However, the retrospective analysis of two multinational large trial comprising 694 patients revealed that 48 weeks of adefovir 10 mg treatment has similar response in decreasing HBV DNA level regardless of genotypes (57).

In 2008, variability in response to adefovir was found in Chinese Han chronic hepatitis B patients (58). Post treatment HBV DNA seroclearance defined as less than 10^3 copies/ml was compared in HBeAg positive chronic hepatitis B, genotype B and C groups (n=183), at 12 weeks, 24 weeks and 48 weeks. Significant difference was reported in 48 weeks post treatment HBV-DNA seroclearance, 41.8% in genotype B group versus 34.6% in genotype C group (P=<0.05). However, in term of ALT normalization and HBeAg seroconverison at 48 weeks, there was no significant result. Other NAs like telbivudine and entecavir are still to be determined in the scope of genotypes. Existing evidence does not show different response. Moreover, it is necessary to explore post-treatment outcome of chronic hepatitis B genotype B and C after NA therapy for the individual geographical region. It is still unknown among Thai patients.

2.10 Previous studies in Thailand

Thailand is an endemic area for hepatitis B. Previous epidemiological studies in Thailand described genotype C is the most prevalent genotype of HBV and other types are type B and mixed (17, 45, 46). Existing evidence has shown the genotype-specific epidemiology of CHB, genotype-specific natural history of the

CHB. Nevertheless, genotype-specific treatment outcome of CHB among Thai patients is not known yet.

2.10.1 Epidemiological surveys

A survey of hepatitis B genotypes among 1231 school Children in Chaing Mai, a province of northern Thailand was genotype C was 90.6%, genotype B 7.5% and mixed 1% among 53 HBsAg positive carriers. In northern Thailand, genotyping in HBsAg positive blood donors also showed predominant distribution of genotype C 89.3% and genotype B 7.4% (45).

There was a nationwide sero-epidemiological survey conducted on 6213 individuals in 2004. In all four major regions of the Thailand genotype C is major genotype of HBV infection. Northern Thailand has genotype C 82%, genotype B 16% and genotype A 2%. Northeastern part of Thailand has genotype C 98 % and genotype B 2%. Southern part of Thailand has genotype C 95 % genotype B 0% and genotype A 5%. The central part of Thailand, area of study current study site has genotype C 70% and genotype B 30 %. The overall result showed genotype C 87.1% genotype B 11.6% and genotype A 1.3% (10). The results of above studies conclusively indicate the predominance of genotype C in Thailand.

2.10.2 Clinical studies

In 2002, there was a study of 107 HBsAg positive cases attending regular follow up at Pramongkutklao hospital. Hepatitis B genotypes and clinical relevance was explored. The result was 25.2% genotype B and 72% genotype C (17). Genotype C group had significantly higher ALT, AST and HBV-DNA level. However, another study reported contradictory result.

Four hundred and seventy patients with chronic hepatitis on long-term follow up at Chulalongkorn Memorial hospital were studied in 2004 genotype C and B groups were compared in chronic hepatitis, cirrhosis and HCC patients. Patients of two genotype groups were compared in 73 HCC patients. The result was no significant difference. No significant difference in clinical parameters was reported between HCC patients of genotype B and C. It was noted that genotype C patients tend to have higher rate of HBeAg positivity. In addition, HBeAg seroconverison rate is slower and the disease course is more severe among Thai patients (46). However, not all of these studies described post- treatment out come in two genotype groups.

Therefore, genotype-specific epidemiology and natural history of CHB in Thailand has been revealed in previous literature. There is still a knowledge- gap about treatment outcome based on genotypes among CHB patients in Thailand. In Thailand genotype C and B are prevalent. Genotype C that has higher risk for cancer and more severe natural course is predominant among Thai patients. Interferon therapy can not treat the severe course of genotype C. However, whether NA widely used in Asia can change the severe natural course of genotype C HBV infection is not well decided. Therefore, it is necessary to know the post-treatment CHB outcome of genotype B and C among Thai patients, especially outcome after widely used NA therapy.

CHAPTER III
MATERIAL AND METHODS

3.1 Study site

The study was conducted at the Hospital for tropical diseases, Mahidol University, Bangkok, Thailand.

3.2 Study design

The study design is retrospective cohort study. Major prognostic factor of interest was chronic hepatitis B genotype. Outcomes of the interest were virological response to antiviral therapy by mean of undetectable viral load, biochemical response by mean of ALT normalization, immunological response by mean of HBeAg seroconversion. Even though the study period is not long enough to detect the occurrence of clinical outcome HCC, the tumor maker AFP was also analyzed.

3.3 Study period

Duration of the study was September 2009 to May 2010.

3.4 Study process

It was retrospective study carried out by reviewing patient record. Record chart of the chronic hepatitis B patients attending or attended at the outpatient clinic, selected according to the inclusion criteria were reviewed after getting permission of director of the Hospital for tropical diseases.

3.5 Ethical issue

The protocol for this project had been approved by ethic committee of the Faculty of Tropical Medicine, Mahidol University, Thailand on 4th November 2009 with the certificate of approval (MUTM 2009-047-01). Reviewing the patient record

charts and data collection was carried out only after getting permission from director of the Hospital for tropical disease, Bangkok.

To protect the privacy of the patients, name of the patients were not included in case record form. Hospital numbers were not directly recorded. Logbook system was used by the help of non-medical person. For the sake of confidentiality, the collected data was kept securely in the personal computer under e-data security system as an encrypted file. The data was kept under the password. Persons who would be accessible to data had signed a confidentiality agreement. Persons who were in the team for data collection had also signed for confidentiality agreement.

3.6 Study population

Chronic hepatitis B patients who has been attending or attended to hepatitis clinic, for follow up visit from 2004 to 2009 with the characteristics described in inclusion criteria were studied. Patient records were reviewed.

3.6.1 Inclusion criteria

Patient's record charts according to the following inclusion criteria were reviewed.

1. Patients diagnosed as chronic hepatitis B by means of HBsAg positivity for more than six months and, presence of HBV-DNA in the serum
2. HBV-DNA level 5 $\log_{10}$ copies per /ml or higher in HBeAg positive cases
3. HBV-DNA level 4 $\log_{10}$ copies per /ml or higher in HBeAg negative cases
4. Patients receiving any kind of nucleoside analogues therapy for the first time will be reviewed, starting from time of getting nucleoside analogue treatment
5. Age between 18 and 70 years
6. Patients infected with chronic hepatitis B genotype B or C

3.6.2 Exclusion criteria

1. Co-infection with HCV (anti-HCV positive)
2. Co-infection with HIV (evidence of anti-HIV antibody positive)

3. Chronic hepatitis B patients who have already acquired HCC, acute liver failure before the treatment

4. Any patient who had ever received any kind of antiviral treatment for chronic hepatitis B previously

The study was well designed to have the study population that could answer the research question. Inclusion and exclusion criteria were applied thoroughly not to include the patients already got the outcome. The time of inclusion was at the start of NA therapy.

3.7 Sample size

Epi Info version 6.5 was used to calculate the sample size. The different response between genotype B and C group was estimated based on a previous study. The cited study result was significant difference in seroclearance of HBV-DNA at 48 weeks post treatment between genotype B and C groups after Adefovir dipivoxil (58). The result of referenced study showed that HBV-DNA seroclearance 48 weeks after adefovir was 41.8% in genotype B patients versus 34.6 % in genotype C patients (P=0.009). Based on cited study result, estimated relative risk 1.4 was used to calculate the sample size. According to previous epidemiological data the area of study site, central part of Thailand has genotype distribution of genotype B 31 % and genotype C 69 % respectively (10).

To compare as 1:2, genotype B 55 cases and genotype C 99 cases would be enough for power 80% and 95% confidence interval. Totally 154 cases would be necessary sample size.

3.8 Data collection

Every selected case record chart was reviewed starting from the time of receiving antiviral treatment. If record is available, the case was followed up for five years and the following data will be collected for every selected case. Data will be collected by using Case Record Form described in appendix.

Age, sex and race of the patients, date of first diagnosis, date of starting nucleoside analogue, type of the nucleoside analogue received were recorded. Pre-treatment HBV-DNA level and HBV-DNA level at sixth month after treatment were

noted. Pre- treatment ALT level and ALT level at sixth month and one year after treatment was be noted. HBeAg positivity or negativity at pre treatment, at six month and one year after treatment will be recorded. AFP level at pretreatment, at sixth month and one year after treatment will be taken. Ultrasound abdomen, MRI, or CT result were also reviewed if available**.**

Data were retrieved from the patient's record chart. Firstly, outpatient list of the liver clinic was followed to search the chronic hepatitis B cases. Chronic hepatitis B cases are also traced by using hospital registry that is kept in the international classification of disease system. Up to this stage, more than 200 case records were reviewed. Among the hepatitis B cases, all the chronic hepatitis B cases that have genotype result were selected. After that, cases were screened out by inclusion and exclusion criteria. Records of the patients currently attending to liver clinic of the hospital for tropical diseases were also traced carefully not to miss the cases.

Many limitations were faced during the collection of data. Hepatitis B genotyping is not a routinely done investigation in every case of chronic hepatitis B. Therefore, limited number of case-records with genotype result had to be searched intensively. Case records with genotype B result were very scanty.

According to the inclusion criteria, carrier cases could not be selected. Some cases having genotype result are inactive carrier. Many case having genotype results were found with the remark no comment for genotype, as viral load is not high enough to detect the HBV genotype. Case record with genotype result other than B and C and, indeterminate result could not be selected.

Some cases have genotype result but already treated by second or third drug. The first time treatment record on some cases cannot be traced up from the registry. In such kind of cases, old data was ordered at the medical record department by special request. Some cases could not be traced back. After meticulous searching of chronic hepatitis B cases eligible to the inclusion criteria and exclusion criteria only forty cases of chronic hepatitis B can was obtained. That was the result of intensive data collection for five months.

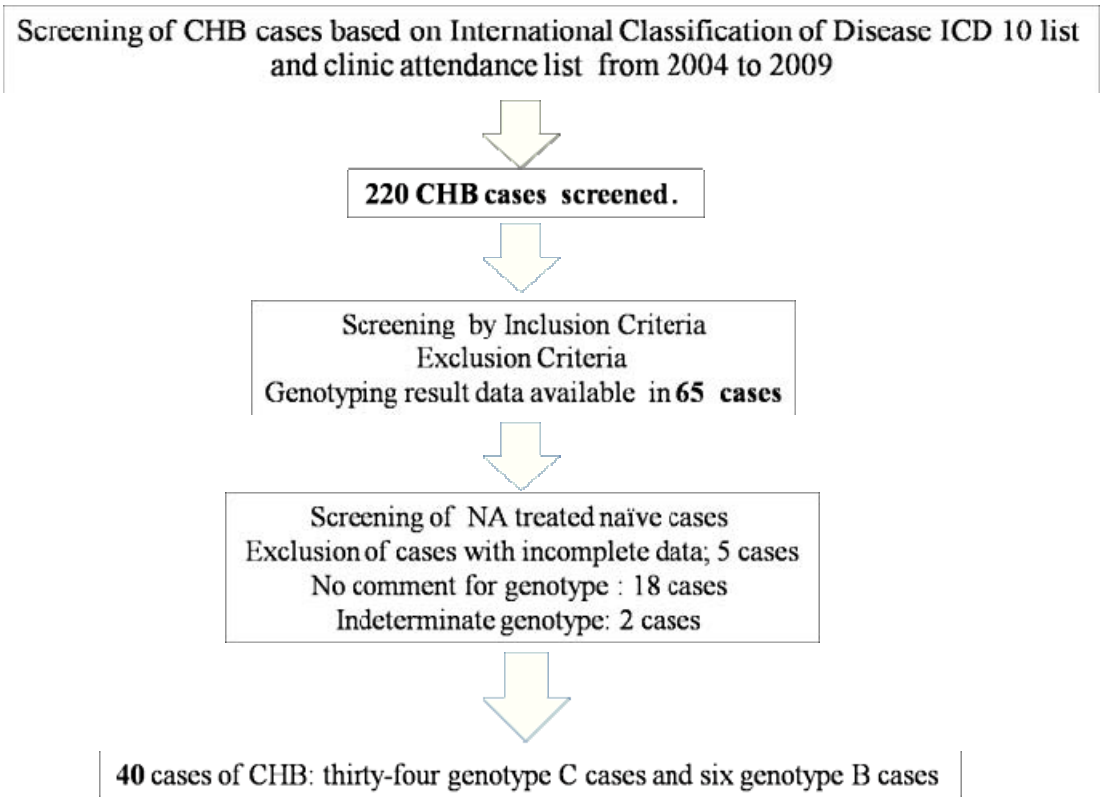


Figure 3.1 steps of data collection

Case record form was used to collect the data .Then the collected data was transferred to Microsoft Excel 2007. A database was prepared by SPSS (Statistical Package for the Social Sciences) version 11.5 software. Validation of the data was done to counter check the data entry. After the data entry, the data was kept under password in the personal computer.

The database was cleaned with the close supervision of adviser. The data was checked case by case, followed up, and rechecked again in case of necessity. It was made sure that selected cases would be the study population that can give the answer to research question.

3.9 Data analysis

Data analysis was done by using SPSS version 11.5. It is the registered version for student at the Faculty of tropical medicine.

3.9.1 Analysis of baseline characteristic in genotype B and C groups

Baseline characteristic data of the two groups was summarized descriptively. The data set contained continuous data as well as categorical data. Categorical data were summarized by percentage. Cross tabulation with chi-square test was applied to compare the categorical data. P value of the Fisher's Exact Test was checked and used to detect the significant difference whenever number in one of the crosstab cells has expected count less than 5 and more than 20 %.

To analyze the continuous variables, the normality of the data was checked by using frequency distribution and histogram with normal curve. One sample Kolmogorov-Smirnov test was used to check the normality of continuous data. Arithmetic mean and standard deviation (SD) were computed to summarize the continuous data if the data was in normal distribution. If the data was not in normal distribution, the result was described as median with minimum and maximum values. Variable with normally distributed data was analyzed to compare means of two groups by using independent sample T -test. Age and viral load $\log_{10}$ copies per ml in the base line characteristic were analyzed by using independent T test. Variables with normal distribution of data like age were compared between two groups by using independent sample T test. ALT, AST level at the baseline was analyzed by using Mann-Whitney U test.

3.9.2 Hypothesis testing

Inferential analysis was used for estimation and testing of hypothesis. All statistical significance was assessed by P value less than 0.05. All tests were two-tailed testing with 95% confidence interval.

Analysis for objective one, two and three were comparison of categorical outcome. Cross tabulation with Fisher's exact test was used to compare the proportion of patients achieved undetectable viral load at sixth month post treatment between genotype B and C groups. The same statistical test was applied for objective two and three.

Analysis for objective four was to compare the mean level of AFP between two groups. The distribution of data was not normal. Therefore, the analysis was done using Mann-Whitney U test to compare the AFP level between two genotype groups at sixth month post treatment.

3.9.3 Additional analysis: description of occurrence of outcome events in genotype B and C groups

Occurrence of outcome event in genotype B and C group was descriptively analyzed. To describe the incidence density, person-year follow up was calculated for achievement of undetectable HBV-DNA level less than 3 $\log_{10}$ copies per ml, ALT normalization and HBeAg seroconversion. By using the follow up period until the occurrence of outcome, Hazard function cures were constructed in SPSS for undetectable HBV-DNA level less than 3 $\log_{10}$ copies per ml, ALT normalization and anti-HBeAb positivity. Moreover, scatter plot of the viral load $\log_{10}$ copies per ml and ALT were constructed for better visualization of individual data and serial changes. Among the ALT normalized group and high ALT group at sixth month, achievement of undetectable HBV-DNA was compared by proportion using chi-square test.

3.9.4 Measure of variable

During the data collection, the methods used for measurement of outcome variables were noted. Methods of HBV genotyping for the study population were noted from the case record, as it is the major prognostic factor of interest in the study.

Currently, there are three available methods up to date namely, sequence analysis, hybridization (line probe Inno-lipa) and microarray. Two kinds of methods were noted in the case record charts. It is found out that Inno-lipa line probe assay was the method used in most of the cases (80%) and other (20%) of the cases had genotype results by sequence analysis (Figure 3.1). Sequencing is considered as gold standard and Inno-lipa has already been proved as comparable to standard (59). Cases with indeterminate or dual genotype results are not included in the study. Inno-lipa method can detect dual infection. According to patient record, Inno-lipa method has limitation for detection of genotype in low viral load cases. Sequence analysis based on gold standard S gene is the back-up method in such situation. However, in this study cases with indeterminate or dual genotype infections were not selected.

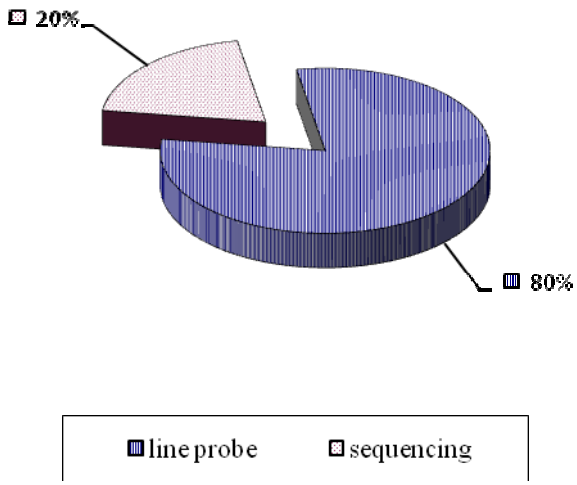


Figure 3.2 Methods of genotyping in study population noted from case record

HBV-DNA viral load detection methods were noted. Three major methods were found in viral load result of the study population. Two methods of viral load tests were noted: COBAS Amplicor Monitor test ($3x10^2$-$2x10^5$ copies per ml) in majority of the test result and Abbott Real time TaqMan HBV (12-110 $x10^6$ IU/ml, 1 IU= 5.82 cp/ml) in few cases. Some record did not have the name of the method but lower limit is of detection was found and noted for viral load results. These methods had different level of minimal and maximal detection. The majority of the undetectable viral load reports were with minimum detection sensitivity less than 1000 copies per ml. Therefore, undetectable viral load in this study was uniformly considered as less than log three copies per ml.

Time of viral load measurement is also observed. In the real clinical practice, the follow up time for viral load is hard to do exactly at sixth month after initiation of treatment. Mean follow duration of sixth month viral load is at 23.47 weeks in the study population. It can be considered as limitation of the study but it can reflect the real clinical practice.

Immunological tests for hepatitis profile were mostly done at laboratory of the hospital for tropical diseases, Bangkok. It was noted that immunological tests for detection of HBsAg, HBeAg, anti-HBe antibody and AFP were done by using Electro-chemiluminescent analyzer. It was noted that ALT and AST measurement were done

at the clinical laboratory of hospital for tropical diseases by using Cobas 501 enzymatic analyzer.

CHAPTER IV
RESULTS

4.1 Baseline characteristic of the study population

The study population was 40 cases of on-treatment chronic hepatitis B cases receiving NA therapy for the first time and comprising six cases infected with genotype B (15%) and thirty-four cases of genotype C (85%). Ethnically, the study population was entirely composed of Thai patients.

Table 4.1 Baseline characteristic of the study population in two genotype groups

Characteristic	Genotype B	%	Genotype C	%	P value
Number of patients	6	15	34	85	
Gender					
male	3	50	23	67.6	0.65^
female	3	50	11	32.4	
Age					
mean	40.67		41.46		0.769#
SD	14.73		11.23		
minimum	23		18		
maximum	63		63		
Ethnicity Thai	6	100	34	100	
Drug					
lamivudine	2	33	12	35	1^$
telbivudine	2	33	11	32.3	
adefovir	2	33	8	23.5	
entecavir$	0	0.	3	8.8	
Viral load log_{10} copies /ml					
mean	6.59		6.53		0.916#
SD	1.6		1.16		

Table 4.1 continued

ALT IU/L pre treatment	**Genotype B**	**%**	**Genotype C**	**%**	**P value**
median	21		60		0.88 [a]
maximum	98		450		
minimum	16		19		
AST IU/L pre treatment					
median	22		47		0.03 [a]
maximum	66		570		
minimum	15		22		
AFP ng/ml pre treatment					
mean	2.4		4.9		0.172[#]
SD	1.33		4.98		
HBeAg pre treatment					
HBeAg + hepatitis	5	83.3	20	64.5	0.641^
HBeAg - hepatitis	1	16.6	14	35.5	
Ultrasound cirrhosis of liver pretreatment					
cirrhotic	0		0		
non cirrhotic	3	50	24	70.6	0.37^
unknown	3	50	10	29.4	
History of alcohol					
present*	0	0	2	5.9	0.207^
not present	6	100	21	61.8	
unknown	0	0	11	32.4	

*exact duration, amount, type, and alcohol could not found in the old record.
^ P-value by Fisher's exact test
[#] P-value by Independent sample T test
[a] P-value by Mann-Whitney U test
[$] calculation of P-value not included entecavir

Study population had 65% male and 35 % female. Male female ratio was 1:1 in genotype B and 2:1 in genotype C group. Mean age in genotype B and C group was not different much. Drug received in two groups is also comparable. In genotype B group, there was no patient treated with entecavir. There was higher percentage of

HBeAg positive hepatitis in genotype B groups than genotype C, but not statistically significant.

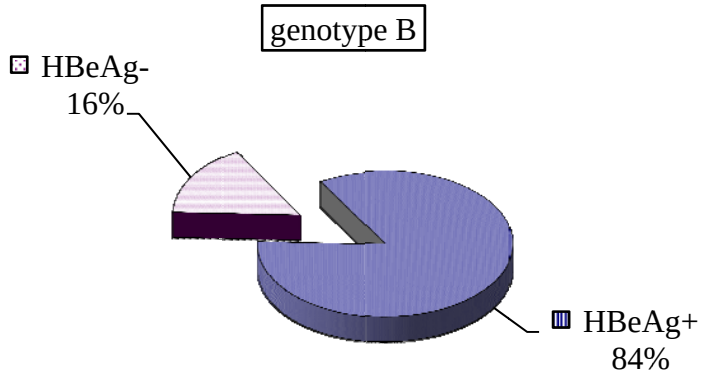


Figure 4.1 Proportion of HBeAg positive and negative chronic hepatitis in genotype B patients

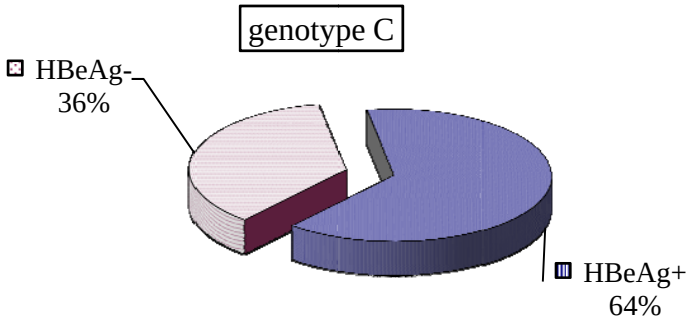


Figure 4.2 Proportion of HBeAg positive and negative chronic hepatitis in genotype C patients

Mean viral load (log $_{10}$ copies per ml) are 6.59 in genotype B group and 6.53 in genotype C group. Considerable difference was observed between base line median ALT level of genotype B and C (21 IU/L vs. 60 IU/L). However, it is not a statistically significant difference (P value 0.88, Man-Whitney U test). Median AST level between two groups was significantly different 22 IU/L in genotype B vs. 47 IU/L in genotype C (P value 0.03, Man-Whitney U test). Median AFP level at baseline were not different significantly.

There was no case of cirrhosis in both groups at the base line. However, three cases in genotype B (50%) and ten cases in genotype C (29.4%) did not have baseline record of abdominal ultrasound before starting antiviral therapy.

Overall, baseline data in two genotypes groups were not different significantly except AST level. Proportion of 2 genotypes in study population and 100% Thai ethnicity is indicating the preponderance of Genotype C among Thai patients.

4.2 Analysis for the objective

4.2.1 Primary objective

According to the primary objective, the proportion of the patients achieved undetectable HBV DNA at sixth month post treatment was compared between genotype B and C infected population.

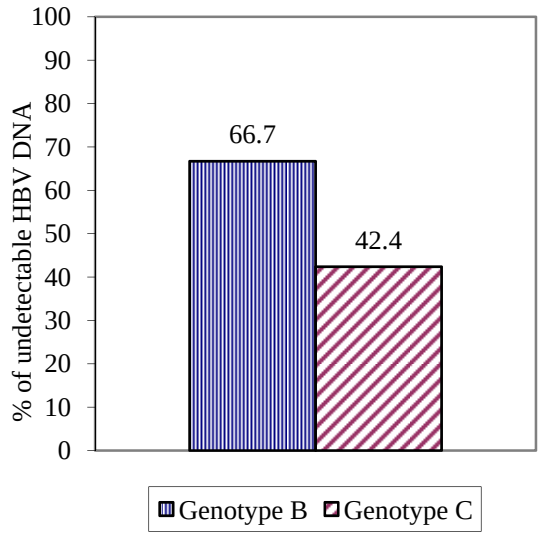


Figure 4.3 Proportion of undetectable viral load in genotype B and C patients after six months of NA therapy

Minimum detectable limit of viral load ranged from less than 1000 to less than 60 copies per ml. In this study, undetectable HBV DNA level could be consistently applied as HBV DNA viral load less than 1000 copies per ml (3 $\log_{10}$ copies per ml).

Table 4.2 Undetectable HBV DNA between CHB genotype B and C at 6th month of NA therapy

CHB N=40	**Undetectable HBV DNA** (18)	**Relative risk (95% CI)**	**P-value**
Genotype B (6)	66.7% (4)	1.57 (0.79-3.14)	0.387
Genotype C (34)*	42.4% (14)	reference	

*****One case was lost to follow up.

Six months after NA treatment, 66.7% of genotype B infected patients achieved undetectable viral load but only 42.7% of genotype C infected patients achieved the undetectable viral load. Genotype B has higher rate of getting undetectable HBV DNA at sixth month than genotype C. Relative risk was 1.57 (95% confidence interval 0.79 to 3.14). P-value by Fisher's exact test was 0.387. Therefore, the difference was not statistically significant.

4.2.2 Secondary objective one

The proportion of patients who achieved ALT normalization, six months after nucleoside analogue therapy was compared between genotype B and C groups. This analysis was done with the ALT normal value less than 30 IU/L for male and less than 19 IU/L for female as defined earlier in chapter three.

Biochemical remission after treatment was compared by normalization of ALT at sixth month after nucleoside analogue therapy. At sixth month of NA treatment, proportion of patients achieved ALT normalization was 50% in genotype B vs. 29.4% in genotype C group. Genotype B group has higher proportion of ALT normalization than genotype C group. Relative risk was 1.7 with 95% confidence interval 0.65-4.42. Genotype B was more likely to get biochemical remission but the difference was not statistically significant. P-value by Fisher exact test was 0.37.

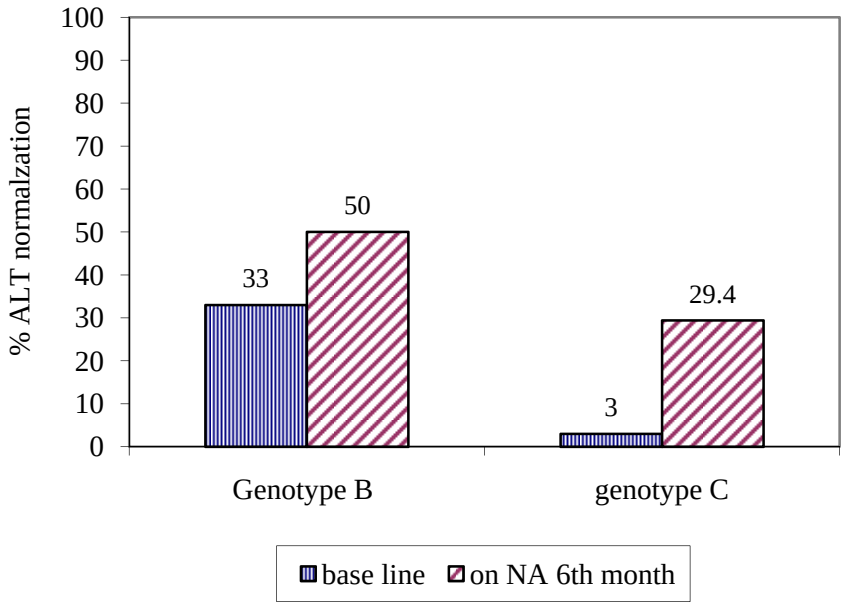


Figure 4.4 ALT normalization in CHB genotype B and C after six months of NA therapy

Table 4.3 ALT normalization in CHB genotype B and C after six months of NA therapy

6th month post treatment N=40	**ALT normalization*** (13)	**Relative risk (95% CI)**	**P-value**
Genotype B (6)	50% (3)	1.7 (0.65-4.42)	0.370
Genotype C (34)	29.4% (10)	reference	

*ALT normal value less than 30 IU/L for male and less than 19 IU/L for female

ALT normal value is still widely used as less than 40 IU/L for both male and female. Therefore, analysis was done with commonly used value even though it was not an objective.

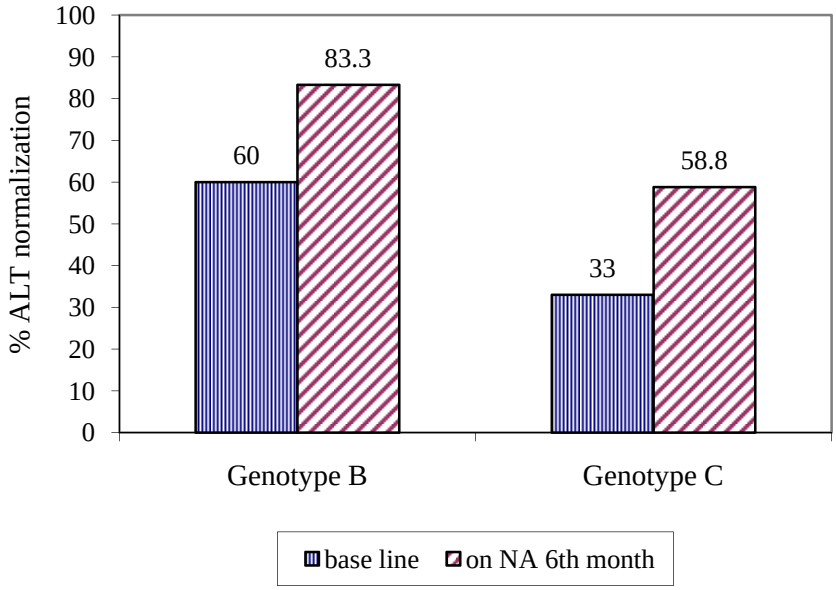


Figure 4.5 ALT Normalization by the normal value less than 40 IU/L after six months of NA therapy in genotype B and C

Table 4.4 ALT normalization in genotype B and C after six months of NA therapy

6th month post treatment N=40	**ALT normalization*** (25)	**Relative risk (95% CI)**	**P-value**
Genotype B (6)	83.3% (5)	1.417 (0.89-2.23)	0.381
Genotype C (34)	58.8% (20)	reference	

*ALT normal value less than 40 IU/L for both male and female

4.2.3 Secondary objective two

HBeAg positive chronic hepatitis B patients comprised the majority of both genotype B and C infected population. Among these patients, HBeAg seroconversion was compared at sixth month after NA therapy.

HBeAg seroconversion was defined as disappearance of HBeAg and appearance of anti-HBe antibody in the serum of a patients diagnosed as HBeAg positive chronic hepatitis B. Serological markers of the two genotype groups was analyzed for HBeAg seroconversion at sixth month of NA therapy. At 6th month of NA treatment, 10% of genotype C infected groups had HBeAg seroconverison vs. nil in genotype B.

Two out of twenty genotype C patients (10%) has attained anti-HBe antibody with the disappearance of HBsAg. Another two patients had both anti-HBe antibody and HBeAg at the same moment. One out of five genotype B patients had anti-HBe antibody but HBeAg had not yet disappeared. None of the genotype B patients had HBeAg seroconversion as defined. Both genotype groups had same proportion 80% with HBeAg still positive and lack of anti-HBe antibody. The spectrum of changes in HBeAg and antiHBe antiboby can be seen graphically in the following figure.

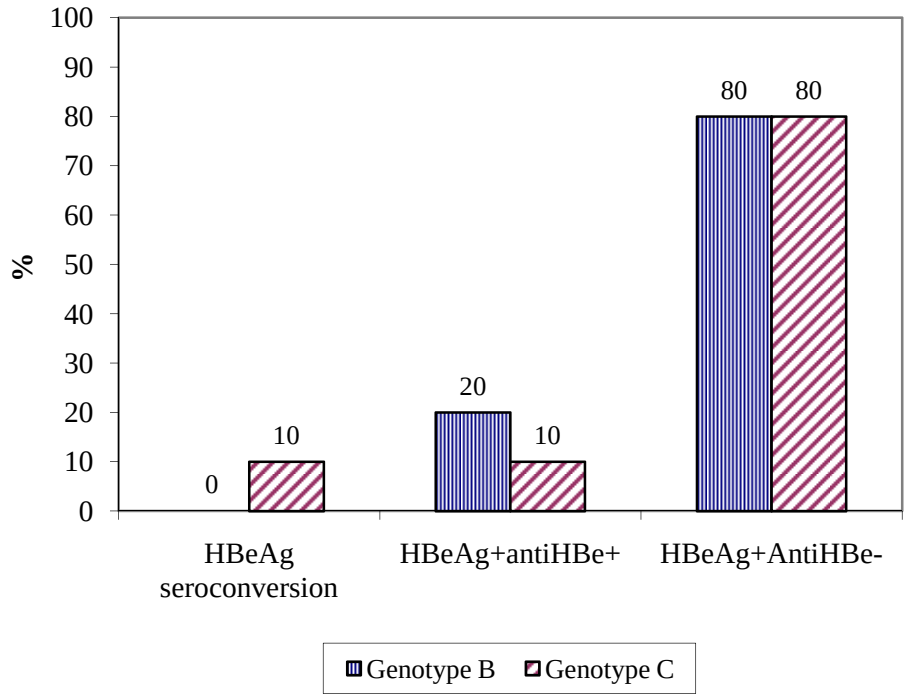


Figure 4.6 HBeAg seroconversion in CHB genotype B and C after six months of NA therapy

Table 4.5 Immunological status of HBeAg positive CHB after six months of NA therapy

HBeAg positive CHB N=25	**HBeAg conversion** % (n)	**HBeAg positive anti-HBe positive** % (n)	**HBeAg positive anti-HBe negative** % (n)
Genotype B (5)	0 (0)	20 (1)	80 (4)
Genotype C (20)	10(2)	10 (2)	80 (16)

4.2.4 Secondary objective three

Level of AFP was compared between CHB genotype B and C infected patients at 6th month of nucleoside analogue therapy.

Table 4.6 AFP level after six months of NA therapy between CHB genotype B and C

6th month post treatment N=38	**Genotype B** (6)	**Genotype C** (32)	**P-value**
Median AFP level (ng/ml)	2.67 (0.85-5.18)	3.05 (1.51-13.60)	0.317

Median AFP levels of two groups were not significantly different. P-value of Mann-Whitney U test is 0.317. It was noticed that all cases in genotype B group had normal AFP at six month but five cases of genotype C had higher than normal AFP.

4.3 Descriptive analysis

4.3.1. Follow up of cases with high AFP and HCC

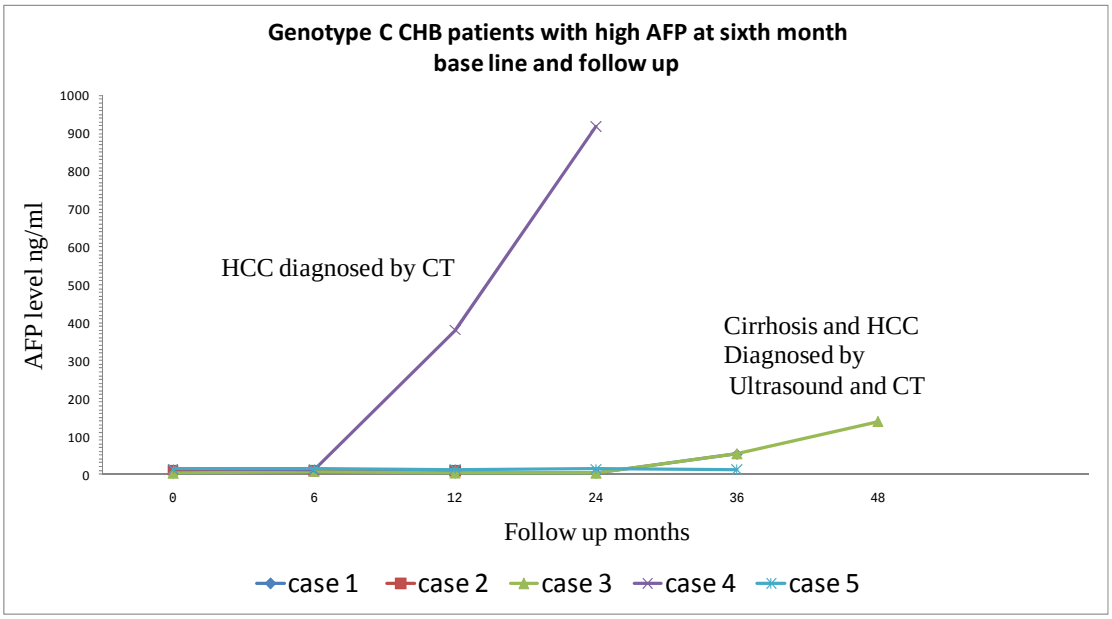


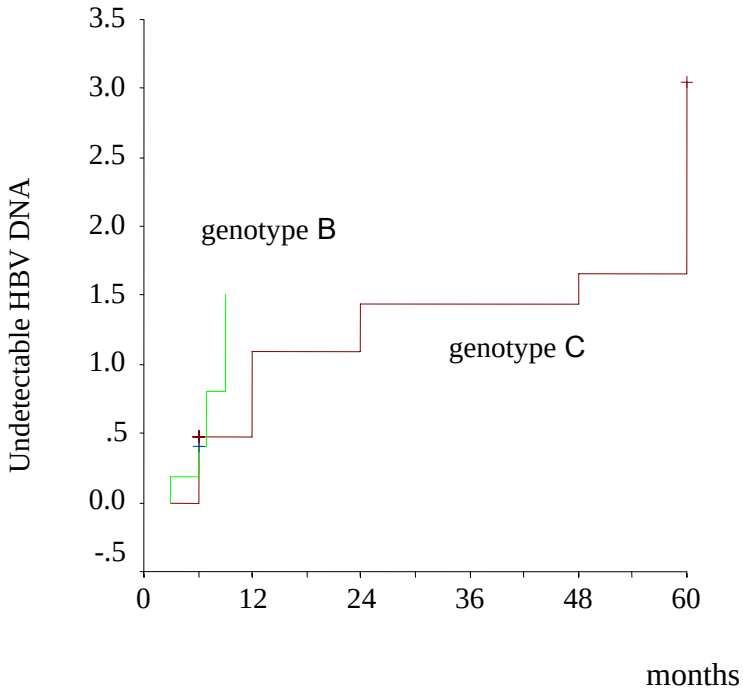
Figure 4.7 Baseline and follow up of genotype C CHB patients with high AFP

Two cases of hepatocellular carcinoma were noted during the follow up of the study population. Both cases were the genotype C infected patients. One case was diagnosed after one year and another case was diagnosed after three years of starting

treatment. Both of the cases were diagnosed during the regular follow up by AFP and abdominal ultrasound. Both of the cases were confirmed and treated case.

4.3.2 Cumulative probability of the outcomes of interest

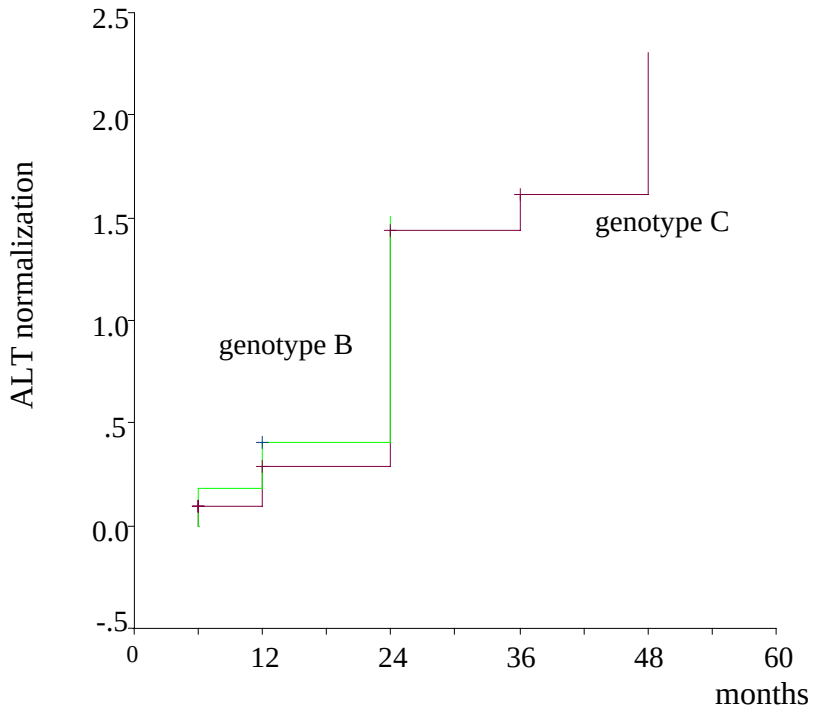
The hazard function curve assists the comparison of the outcome occurrence over time by visualization**.** Figure 4.8 is hazard function curve for cumulative probability of undetectable viral load in genotype B and C. It is a descriptive analysis. Genotype B curve is more on the left side to the Y-axis than the curve for Genotype C. Difference in cumulative probability of achieving undetectable viral load in two genotype groups over time can be seen. Within the twelve month after treatment, the curve for cumulative probability of genotype B to achieve the undetectable viral load is visually higher than that of genotype C. Notable point is that current study cannot cover the aspect of change in drug after the first NA. These curves can be only the descriptive finding of time and occurrence of outcome in study population. The table below the curve showed the event of undetectable HBV-DNA with the denominator of remaining number at each time interval of follow up.



months	6	12	24	36	48	60
B (6)	4/1	0/1	0/1	1/0		
C (34)	14/19	12/6	2/2	0/2	0/2	1/0

Figure 4.8 Cumulative probability of undetectable HBV DNA in CHB genotype B and C

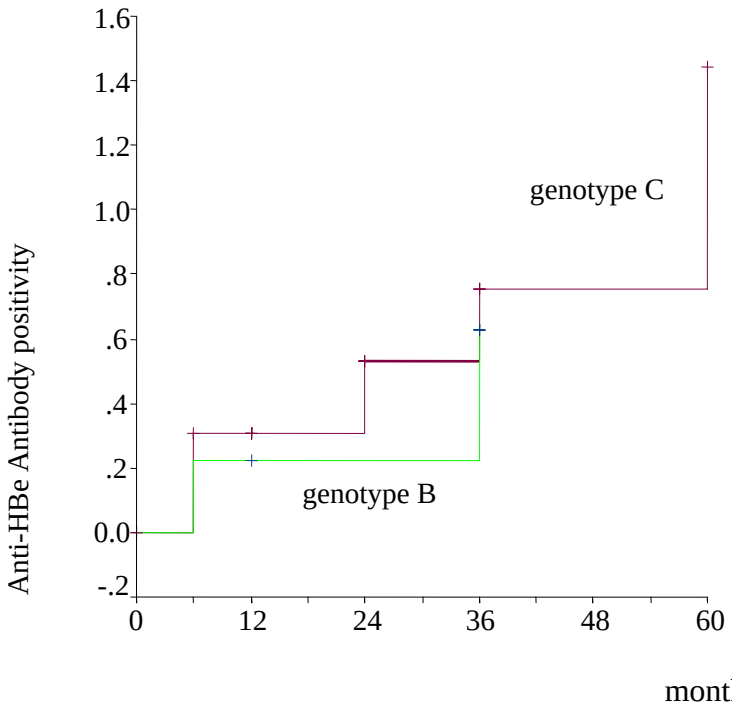
Cumulative probability of sustained biochemical remission by means of ALT normalization can be seen in the figure 4.9. Number of events at each interval of follow up months is shown in table under curve with the denominator of remaining cases at each interval of time. The cumulative probability of sustained ALT normalization is visually higher in genotype B group than the genotype C group until twenty-four month follow up.



months	6	12	24	36	48	60
B (6)	1/5	1/3	2/1	0/1	0/1	1/0
C (34)	3/28	5/22	15/6	1/4	2/2	2/0

Figure 4.9 Cumulative probability of biochemical remission in CHB genotype B and C by ALT normalization (Normal value of ALT is less than 40 IU/L)

Cumulative probability of HBeAg seroconversion can be seen in the hazard function curve in figure 4.10. These curves are just an attempt to show the descriptive finding of occurrence of event of interest over time. In this analysis, HBeAg sero-conversion event was not completely fit with definition of HBeAg sero-conversion in current guidelines. Event of anti HBe anti body appearance were noted, as event as shown in table below figure 4.10 with denominators were number remaining at each time interval of follow up**.** The curve for anti-HBe antibody response in genotype B and C is somewhat overlapped at six month to twelve-month duration.



months	6	12	24	36	48	60
B (5)	1/4	0/3	0/3	1/0		
C (20)	5/13	0/10	2/5	1/2	0/2	1/0

Figure 4.10 Cumulative probability of appearance of anti-HBe antibody in CHB genotype B and C

4.4 Descriptive analysis emphasizing individual values

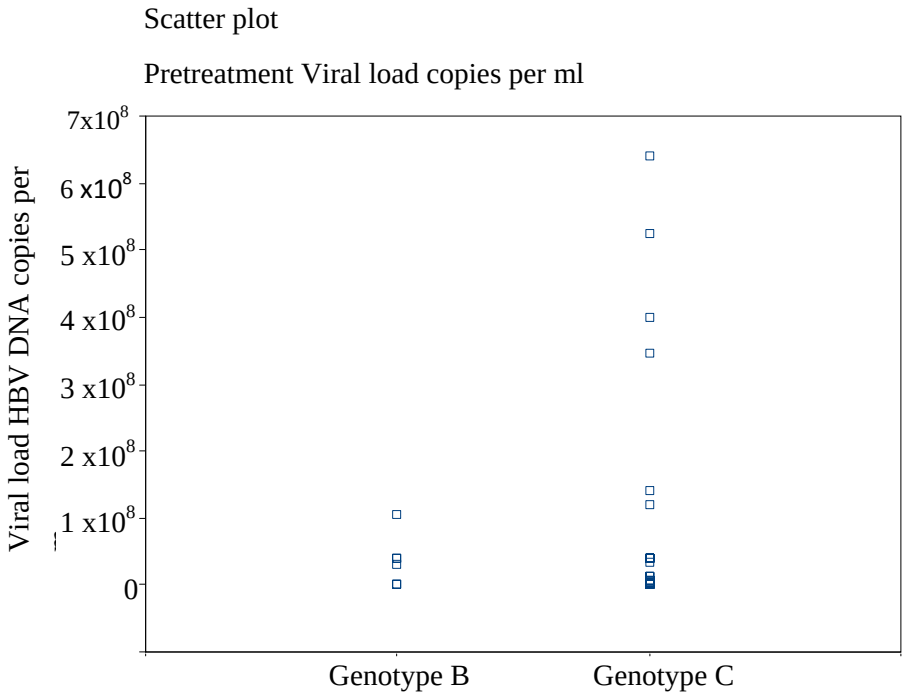


Figure 4.11 Scatter plot diagram of pretreatment viral load copies per ml in genotype B and C

The real value of viral load copies per ml can be seen in two genotype B and C groups. Pre- treatment viral load in two groups of genotypes are different obviously when the value are seen in scatter plots. The following are the log values in serial changes.

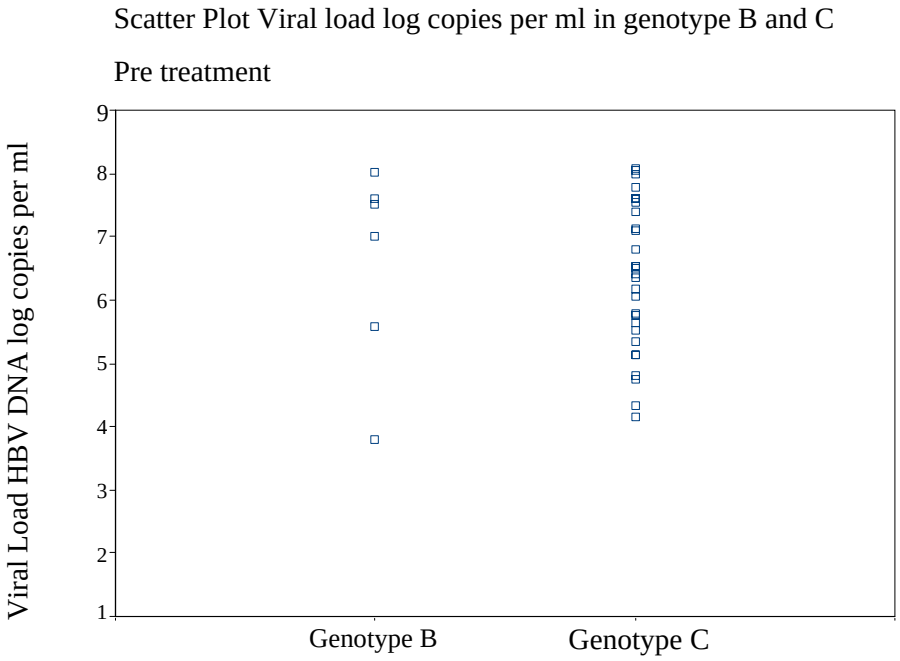


Figure 4.12 Scatter plot diagram of pretreatment viral load log copies per ml in genotype B and C

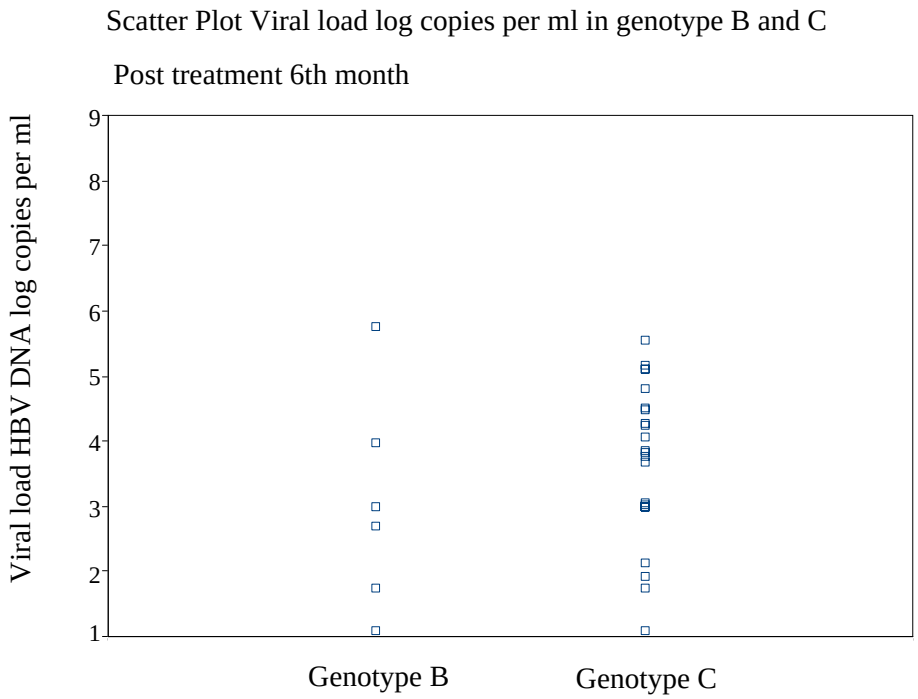


Figure 4.13 Scatter plot diagram of viral load log copies per ml in genotype B and C at 6th month post treatment

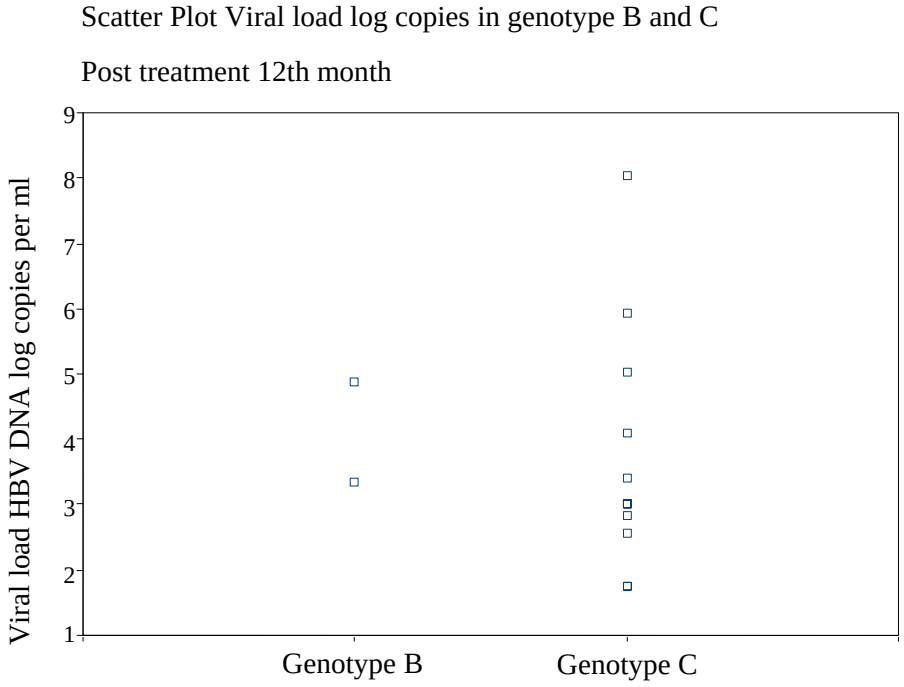


Figure 4.14 Scatter plot diagram of viral load log copies per ml in genotype B and C at 12th month post treatment

Scatter plot of the ALT

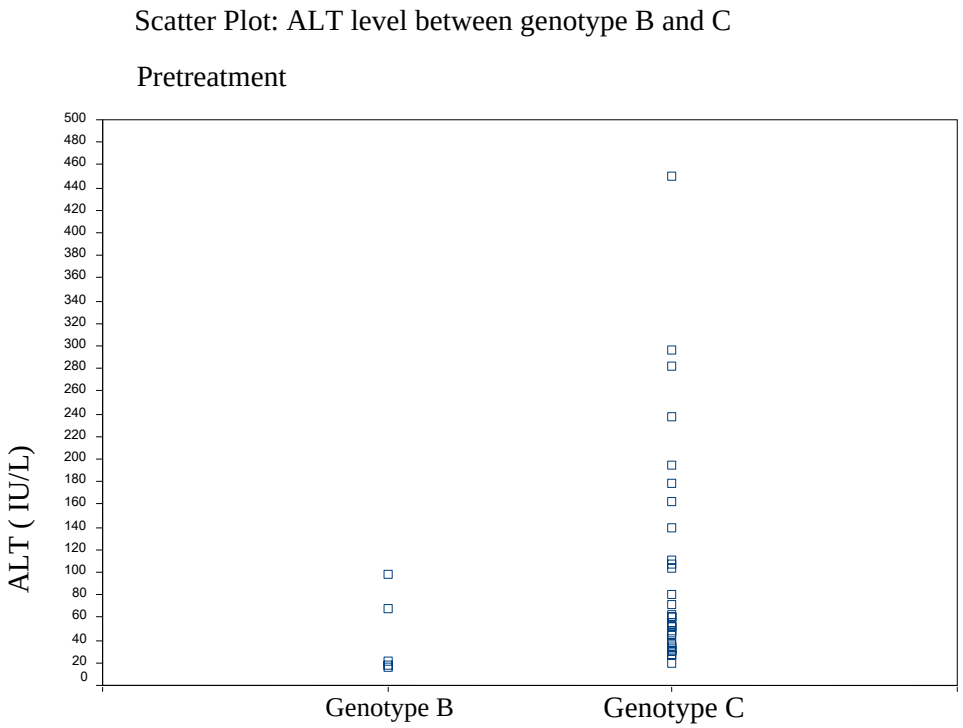


Figure 4.15 Scatter plot of ALT (IU/L) in genotype B and C at pre-treatment

Scatter Plot ALT level between genotype B and C

Six months post treatment

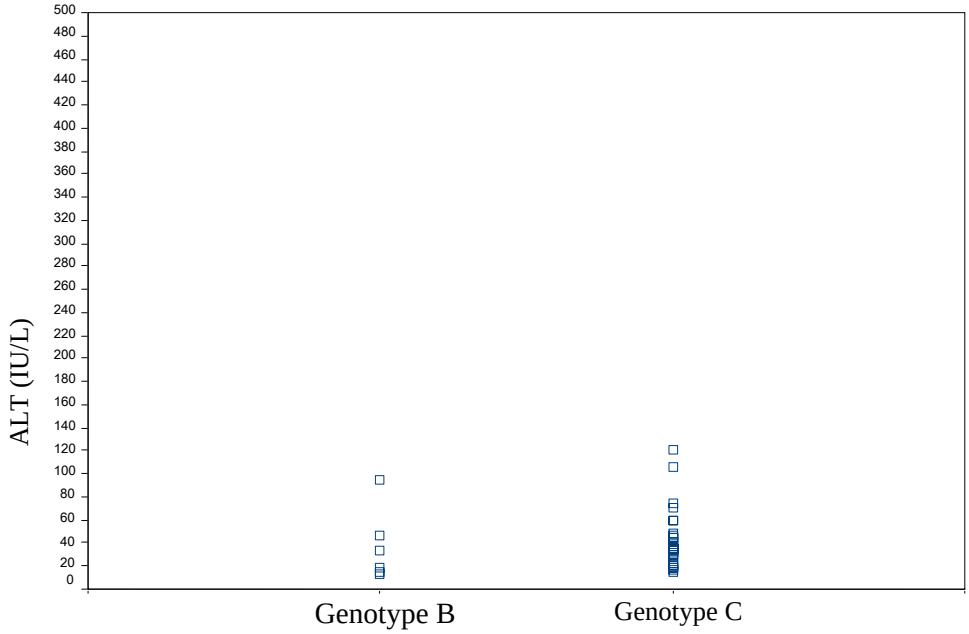


Figure 4.16 Scatter plot of ALT (IU/L) in genotype B and C at 6th month post treatment after NA therapy

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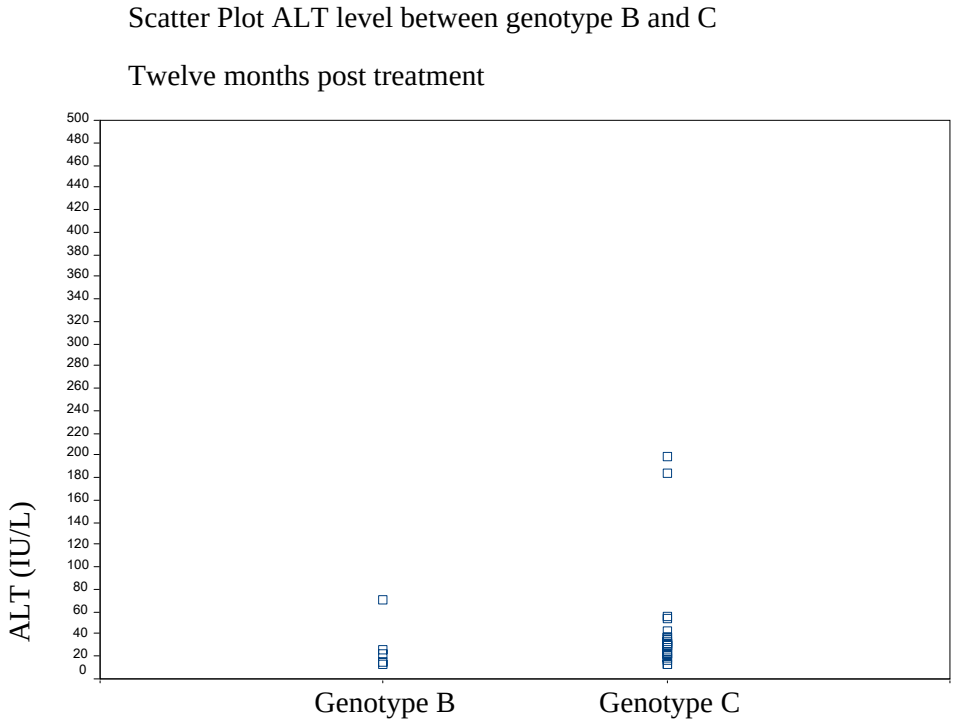
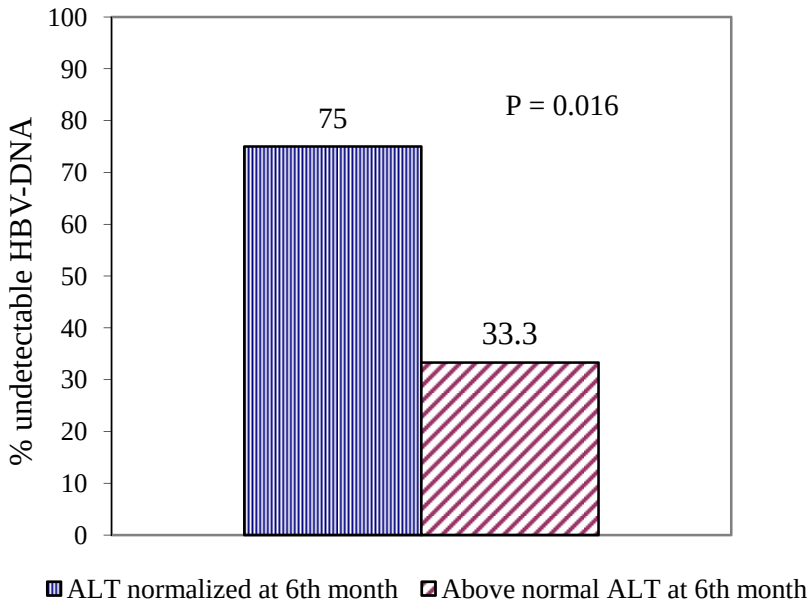


Figure 4.17 Scatter plot of ALT (IU/L) in genotype B and C at 12th month post treatment after NA therapy

The scatter plots are showing the serial changes in the value of viral load and ALT as a whole group and individual values. HBV DNA values before and after treatment can be seen clearly. Decline in ALT level can be seen as a whole group.

4.4 Additional analysis and significant finding

An observation was done to look at the whole study group as two groups: those with ALT normalization at six month and those without ALT normalization at six month after NA therapy. A significant difference was found between these two groups to achieve undetectable HBV DNA at sixth month. Analysis was done using both currently used normal value as 40 IU/L and new normal value of ALT suggested by current guidelines: less than 30 IU/L for male and less than 19 IU/L for female.



(Normal value of ALT cut off points was < 30 IU/L for male and < 19 IU/L for female)

Figure 4.18 Achievement of undetectable HBV DNA at sixth month post treatment in ALT normalized group and higher than normal ALT groups at sixth month of NA therapy

With the new cut off point of normal ALT level, 75% of ALT- normalized patients achieved undetectable HBV DNA at sixth month of NA therapy vs., 33.3 % of patients with high ALT achieved undetectable HBV DNA. ALT normalized group is more likely to achieve undetectable viral load than high ALT group (RR= 2.25 with the 95% confidence interval 1.20 to 4.21). P value by Pearson Chi- square test is 0.016**.**

Table 4.7 Achievement of undetectable viral load in ALT normalized and high ALT group at six month after NA therapy

CHB N=39	**Undetectable HBV DNA (18)**	**Relative risk (95% CI)**	**P-value**
ALT normalized* (12)	75% (9)	2.25 (1.20 to 4.21)	0.016
Above normal ALT (27)	33.3% (9)	reference	

*Normal value of ALT cut off point: male < 30 IU/L, female < 19 IU/L

The same analysis was done with ALT normal value at 40 IU/L as it is used in clinical practice commonly.

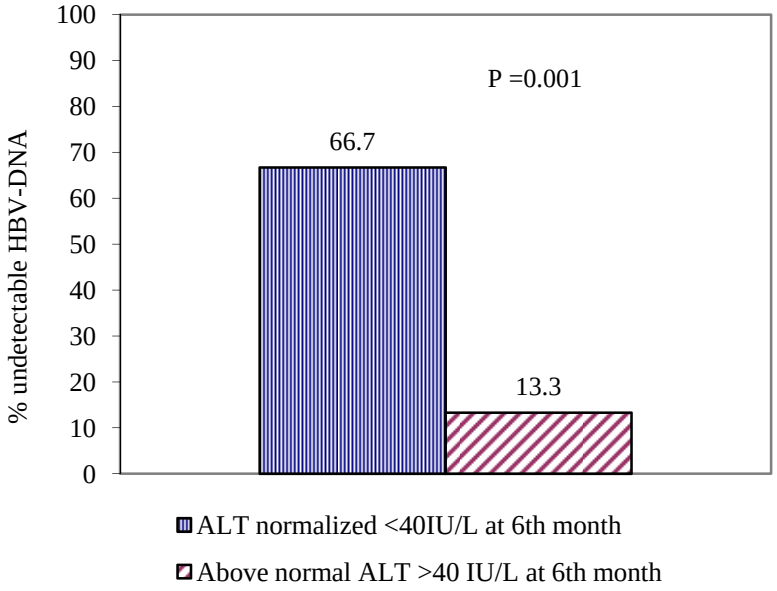


Figure 4.19 Achievement of undetectable HBV DNA at sixth month post treatment in ALT normalized group and high than normal ALT groups at sixth month, (Normal value of ALT cut off point was < 40 IU/L for male and female)

Table 4.8 Achievement of undetectable viral load in ALT normalized and high ALT group at six month after NA therapy

CHB N=39	**Undetectable HBV DNA (18)**	**Relative risk (95% CI)**	**P-value**
ALT normalized* (24)	66.7% (16)	5 (1.34-18.73)	0.001
Above normal ALT (15)	13.3 % (2)	reference	

*ALT cut off normal value < 40 IU/L

Significant difference was revealed between normal ALT group and high ALT group to get the undetectable viral load at sixth month (relative risk 4.333 with 95% confidence interval 1.174-15.994. P value by Pearson Chi- square was 0.003. Therefore, result of above two analyses can be summarized as followed. Achievement of undetectable viral load at sixth months is higher in post-treatment ALT normalized

patients than in high ALT patients. It is applicable to chronic hepatitis patients receiving first time of NA and genotype B and C patients.

CHAPTER V
DISCUSSION

Currently, there are two types of therapy for chronic hepatitis B: interferon therapy and NA therapy. NAs are widely used in Asian counties also in Thailand (60). There are eight known genotypes of hepatitis B (A-H) variably present in different geographical location and ethnicity. Thailand has two common HBV genotypes, B and C, with the majority being genotype C.

It is an interesting question whether genotype B and C are different in term of treatment outcome after NA therapy. Previous studies in different population had given the different answers. Previous studies in Thailand had revealed the different natural course between HBV genotype B and C infection (17). Our study result is expected to assist the practicing physicians for prediction of the treatment outcome of NA therapy at sixth month post treatment in the scope of HBV genotypes.

Forty cases of chronic hepatitis B comprising six cases of genotype B and 34 cases of genotype C were included in the analysis. All cases in study population were carefully selected with inclusion criteria, and all were treatment naïve patients received the NA for the first time. All were Thai patients.

5.1 Genotype impact on treatment outcome of CHB

Current study had found that the proportion of patients achieved undetectable HBV-DNA were not different significantly among genotype B and C at sixth month of NA treatment. The proportion of ALT normalization and HBeAg seroconverison were also not significantly different between genotype B and C infection at sixth month of NA therapy. Tumor maker AFP levels between genotypes B and C were not significantly different at sixth month. As a summery, among Thai patients with CHB, genotype B and C are not different significantly for the treatment outcome at sixth month of NA therapy for virological outcome, biochemical remission, HBeAg seroconversion and level of tumor maker.

Result of current study is worthwhile to be compared with the context of previous studies. Wiegand, Hasenclever et al. 2008 had reported the combined analysis of available evidence of treatment outcome by HBV genotypes (61). Their analysis included the published studies of sample size above 30 with different kinds of NA treatment and different outcome measures. Data from three randomized trials and five observational studies were included in their analysis to compare genotype B vs. C and A vs. D HBeAg positive hepatitis. Three studies comprising two trials and one observational study on treatment outcome of lamivudine therapy were included in their analysis for HBeAg negative patients. The finding of that combined analysis revealed that treatment response was not different between genotype B and C. That analysis did not contain Thai ethnicity. Our study result is concurring with result of that combined analysis and adding up the scientific evidence with Thai ethnicity.

In our study, HBeAg conversion between two genotypes was not different significantly. The number in HBeAg positive hepatitis subgroup was small to claim the finding and the time for outcome measure, six months might be not long enough to see HBeAg conversion in most of the cases. Genotype C group has 10% HBeAg conversion. Genotype B group did not have any case of HBeAg seroconverion meanwhile 20% of the genotype B had both HBeAg and anti-HBe antibody. Chan, Wong et al. 2003 had reported that hepatitis B genotypes had no impact on HBeAg seroconversion after lamivudine therapy (21). Their prospective study in Hong Kong had 35 patients on lamivudine and 96 controls. The author demanded the study in other area and ethnic groups. Current study result in Thai patients is agreeing with result in Hong Kong population.

It is interesting that two clinical trials of the same NA in different location and ethnic groups reported different result about the genotype and treatment outcome. Zeng, Deng et al. 2008 had reported the difference in HBV DNA reduction at 48 weeks post-treatment by adefovir therapy between CHB genotype B and C in Chinese Han population (58). Comparison was by mean of percentage of HBV DNA level reduction to less than $\log_{10}$ 3 copies per ml. Sample size was 183. It is worth mentioning that 24 weeks response was not different in two genotype groups.

On the other hand, Westland, Delaney et al. 2003 reported that reduction in HBV DNA after 48 weeks of adefovir dipivoxil 10 mg was not different among

genotype B and C (57). It was a multinational trial of 694 participants at analysis. In that multinational trial, patients from Thailand were also enrolled to the study (57). The author raised the question of predominant genotype in each country and relation between genotype and clinical response.

There are many things to consider at this point. Even bigger study with the same agent of NA revealed negative result in multi-ethnicity and positive result in study population entirely composed of Chinese Han population. In current study, host and pathogen factors are fixed as Thai ethnicity and HBV genotype B and C. Therefore, the context of current study is of the same opinion with suggestion of previous prospective randomized controlled trials.

It is likely that the treatment response of genotype B and C are not different after NA therapy despite the different natural history of two genotypes B and C. However, the result of current study can be applied only up to six-month post treatment. Long-term treatment outcome should be explored in future research.

5.2 Considering baseline characters of two genotype groups

Base-line characters of the study had revealed notable points. The study population was 40 cases of treatment CHB patients. Among 40 CHB cases, there were 6 cases of genotype B and 34 cases of genotype C. Larger number of genotype C and 100% Thai ethnicity of all the population at a single study site in Bangkok are indicating the obvious epidemiological preponderance of genotype C in the study area. In other areas of Thailand, genotype C has even higher proportion according to previous study report. Suwannakarn, Tangkijvanich et al. 2008 reported prevalence of genotype C as 70% to 95% depending on the area of Thailand (10). Base line data on genotype distribution in current study is agreeing with the previous literature.

Study population as a whole has higher male proportion. Genotype B group had equal number of male and female, meanwhile genotype C has higher male proportion than female. It is similar to gender distribution in previous papers.

In both groups, mean age was similar, age (years) in genotype B was 40.67 and in genotype C 41.46. It is also notable finding that mean age of study population in both genotype are older than mean or median value age (years) in studies of other countries for example, China: genotype B 31.6, genotype C 33.1 , Taiwan: genotype B

30.4, genotype C 34.9, Hong Kong: genotype B 34 and genotype C 29 respectively (21, 23, 58). Another Thai study about the natural history of hepatitis B genotype also showed mean age 47.2 in genotype B and 43.8 in genotype C (17). Study population in our study is treatment naïve patients. So, the mean age can reflect the time of consultation, time of starting treatment or age at initial diagnosis. Maeshiro, Arakaki et al. 2007 has reported age specific natural course of chronic hepatitis B. After fourth decade of life, genotype C is a significant predictor for development of liver cirrhosis (44). After age 40 cirrhosis of liver is also not rare in HBeAg negative genotype B, chronic hepatitis.

ALT and AST are two major enzymes reflecting the liver cell injury during dynamic pathological process in chronic hepatitis B. In the two groups of study population , Median ALT value in genotype B group at base line was 21 IU/L but, genotype C group revealed higher ALT median value 62 IU/L. It is a clinically notable finding despite lack of statistical significance. The difference is clearer in scatter plot of base line ALT values in figure 5.13. Genotype C group also had significant higher median AST value at the base line. Previously, Suwannakarn, Tangkijvanich et al. 2008 described the higher liver enzymes in the natural history of genotype C infection in Thai patients (10). Base line biochemical data in our study is adding up the strength of existing evidence.

Clinically, there are two major types of chronic hepatitis B based on HBeAg positivity well known as HBeAg positive and HBeAg negative chronic hepatitis B. Our data at base line showed genotype B infected groups had higher proportion of HBeAg positive CHB. It is different from data of previous study in Thailand. Jutavijittum, Jiviriyawat et al. 2006 reported higher proportion of HBeAg positive hepatitis in Genotype C (45).

In the base line data of current study, two genotypes groups had comparable mean viral load by log value, $\log_{10}$ copies per ml. It is also worthwhile to see the viral load scatter plot in real copies per ml in figure 4.11. Genotype C infected group contained a few cases of extremely high viral load at base line. This finding is also similar to previous study result of natural history of CHB genotypes in Thailand (46).

Most of the previous study on treatment outcome of CHB genotypes had only one drug. Another design was analysis of clinical trial data in the view of two genotype groups. In current study, there were four kinds of NA received by the patients namely, lamivudine, telbivudine, adefovir and entecavir. It was reasonably questionable whether effect of different drugs may have effect on outcome. The newer generation entecavir has better efficacy than the other drugs. Only 8.8% of the patients in genotype C have received entecavir but no patient was treated with entecavir in genotype B group. Considering other three drugs, the distribution of NA between the two-genotype groups was comparable and not different statistically. However, these drugs have different efficacy for achieving sustained virological response especially on long -term, 48 weeks onward based on trial results (13). During the relatively short follow- up period of six months, effect of different drugs might not modify the primary outcome because of the short duration and treatment naïve population. It may be reflecting logistic circumstances in real clinical practice.

5.3 Recommendation based on descriptive findings

Considering all the findings described above, the base line data of the current study is much similar to the previous report in study of natural history. The result on hypothesis testing revealed that treatment outcome of genotype B and C sixth month after NA therapy is not different significantly between genotype B and C. However, CHB is a disease demanding long-term treatment to prevent the long-term clinical outcome. There are evidences in the previous literature for difference in genotype -specific natural history. It is of the great interest whether antiviral can change the genotype specific natural course of the disease on long-term.

Result of descriptive analysis has showed cumulative hazard curves suggesting that there can be difference treatment outcome on long- term between genotype B and C. However, our study population is not big enough to predict the long-term course in sub-groups. Therefore, it was merely an attempt to describe or generate the hypothesis. The study population of treatment naïve patients can no longer be simply on one single drug after one year or more on treatment. After duration, about one year the NA treated naïve population will split into two or more populations based on drug resistance and strategy of change in drug. Resistance is

common especially with lamivudine. Study with sample size large enough for the subgroup analysis based on different strategy of changing drugs and longer period of follow-up will be necessary for prediction of genotype-specific long-term outcome in chronic hepatitis B. It is the recommendation for future research based on descriptive finding of current study.

5.4 Other significant finding; correlation between undetectable HBV-DNA and on-treatment ALT normalization

It is worthwhile to observe the whole group in the view of biochemical remission. It was found that achievement of HBV- DNA level is significantly different between post treatment ALT normalized group and post treatment high ALT group at sixth month (Table 4.7). The result is also significant with analysis by newly set lower normal value ALT (Table 4.8). This result will be confined to treatment naïve patients receiving NA especially genotype B and C CHB in Thailand. In practice, measurement of serial viral load is expensive. In the setting of practice in developing countries, this finding would be assisting the physician to predict the virological outcome by ALT normalization at sixth month. It is five times likely to get the undetectable viral load in ALT normalized groups at sixth month post treatment (RR=5 with 95% confidence interval 1.34-18.73).

5.5 Limitation

There were many limitations along the process of this research. As this was an academic study, there was constraint of time and financial resource. The main limitation was availability of the cases. At the time of proposal, it was well thought out for feasibility of the research. Methodologically the study was well designed to have the proper study population. In the reality to get the data when the patient started NA as a naïve patient was also very difficult. At the older time, viral load may be not affordable to check regularly for every case. The time of viral load cannot be exactly as six monthly as researcher needs. If eligible case does not have pre- treatment and base line viral load, the case cannot be selected again.

Availability of genotype result was main determinant to select the case. Genotyping is not yet a routine test for management of CHB. Some cases having genotype results in patient record charts are with the answer of low viral load and undetermined genotype. Some cases having genotype result are still inactive carrier. Genotype B cases are very scanty among the record. It may be because of actual epidemiological pattern at the study site. All these factors made us difficult to get the adequate sample size within limited time -frame. Nevertheless, the help and support of all the teachers was supportive and crucial at all steps of the research.

The primary outcome of the current study is not an ideal end-point in the treatment of chronic hepatitis B. However, it has documented importance. Virological response at sixth month (24 weeks) has value for decision of partial virological response especially with the drugs of moderate potency and low genetic barrier to resistance like lamivudine and telbivudine (13). The result of our study is expected to provide useful evidence for practicing physician at an important time point in treatment of chronic hepatitis B.

CHAPTER VI
CONCLUSION

The aim of current study was to find out the six months post treatment outcome of chronic hepatitis B genotype B and C receiving NA therapy among patients in Thailand. The answer to our research question was genotype B and C are not different at six months post NA treatment in term of virological, biochemical and immunological outcomes. This result will add up the existing evidence of genotype-specific epidemiology and natural history of chronic hepatitis B in Thailand with treatment outcome prediction. This result may provide some clues for the practicing physicians to think about the prognosis of CHB.

There have been many advances in understanding and evidence for chronic hepatitis B in recent years. However, clinical research challenges remain for optimal management of chronic hepatitis B with uncertainty in many areas. It is believed that not only the main result and findings, the sharing of experiences and lessons learnt though the whole research process may contribute the clues for hepatitis B research in future.

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APPENDIX

Case Record Form

Date of data collection ☐☐/☐☐/☐☐

Recoded case number ☐☐☐☐☐☐☐☐☐	
History	
Age ☐☐	Date of diagnosis ☐☐/☐☐/☐☐
• *To **tick** ✓the appropriate box for the each item*	
Sex ☐1 male ☐0 female	Race ☐1 Thai ☐0 non-Thai
Duration of exposure	☐1 known ☐ 0 not known
Family history of hepatitis B	☐1 present ☐ 0 absent
Blood transfusion	☐1 present ☐ 0 absent
Tattoo	☐1 present ☐ 0 absent
Alcoholics	☐1 present ☐ 0 absent
Health care worker	☐1 yes ☐ 0 no
Drug	
Date of starting drug ☐☐/☐☐/☐☐	
• *To **choose the number** respective to drug in patient record chart fill the cell with number*	

Lamivudine =1 Telbivudine =2 Adefovir =3 Entecavir =4	Drug received ☐	Dose ☐☐☐ mg ☐ times per day

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Virological markers				
HBV Genotype *to tick ✓ B or C*				Date of result
Genotype B □1 Genotype C □2				□□/□□/□□
HBV DNA Viral load				
	Log_{10} copies/ml	copies/ml	Undetectable HBV DNA *To tick✓ yes or no*	to specify the undetectable level of the test
Pre treatment	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
Sixth month	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
One year	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
Two year	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
Three year	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
Four year	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	
Five year	□□.□□	□□□□□□□□	□1 Yes □2 No	□□□□
Date of the result			□□/□□/□□	

IMAGING	***To tick✓yes or no***			
	USG		**CT**	**MRI**
	Cirrhosis	**HCC**	**HCC**	**HCC**
Pre treatment	□1Yes□2 No			
Date of result	□□/□□/□□			
6 month	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□
1 Year	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□
2 Year	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□
3 Year	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□
4 Year	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□
5 Year	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No	□1Yes□2 No
Date of result	□□/□□/□□	□□/□□/□□	□□/□□/□□	□□/□□/□□

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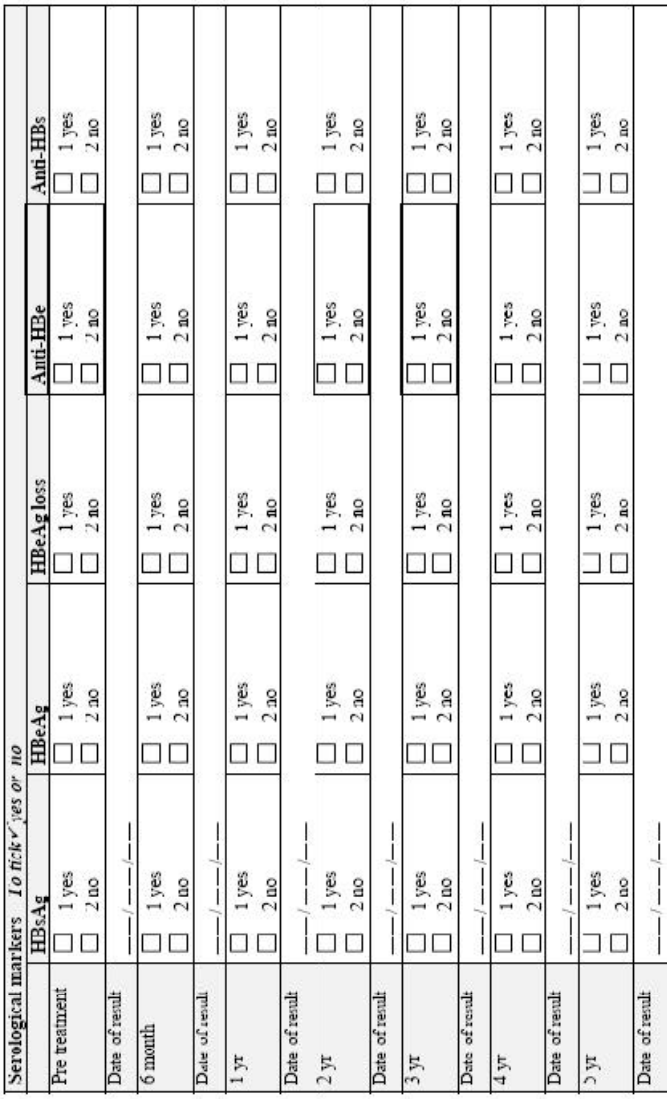
IMAGING *To tick✓yes or no*				
	USG	*To tick✓*		
	Fatty liver	**Mild 1**	**Moderate 2**	**Severe 3**
Pre treatment	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
6 month	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
1 Year	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
2 Year	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
3 Year	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
4 Year	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			
5 Year	□1Yes□2 No	□	□	□
Date of result	□□/□□/□□			

CRF page 5



Serological markers *To tick ✓ yes or no*				
	HBsAg	**HBeAg**	**HBeAg loss**	**Anti-HBe**	**Anti-HBs**
Pre treatment	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
6 month	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
1 yr	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
2 yr	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
3 yr	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
4 yr	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				
5 yr	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no	☐ 1 yes ☐ 2 no
Date of result	___/___/___				

CRF page 6

Biochemical Tests

	ALT IU/ml	≤normal ALT *To tick: yes or no*	AST IU/ml	≤normal AST *To tick: yes or no*	AFP ng/l	≤normal AFP *To tick: yes or no*
Pre treatment	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
6 mth	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
1 yr	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
2 yr	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
3 yr	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
4 yr	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	
5 yr	□□□□	☐ 1 yes ☐ 2 no	□□□□	☐ 1 yes ☐ 2 no	□□□□.□□	☐ 1 yes ☐ 2 no
Date of result	--/--/--				--/--/--	

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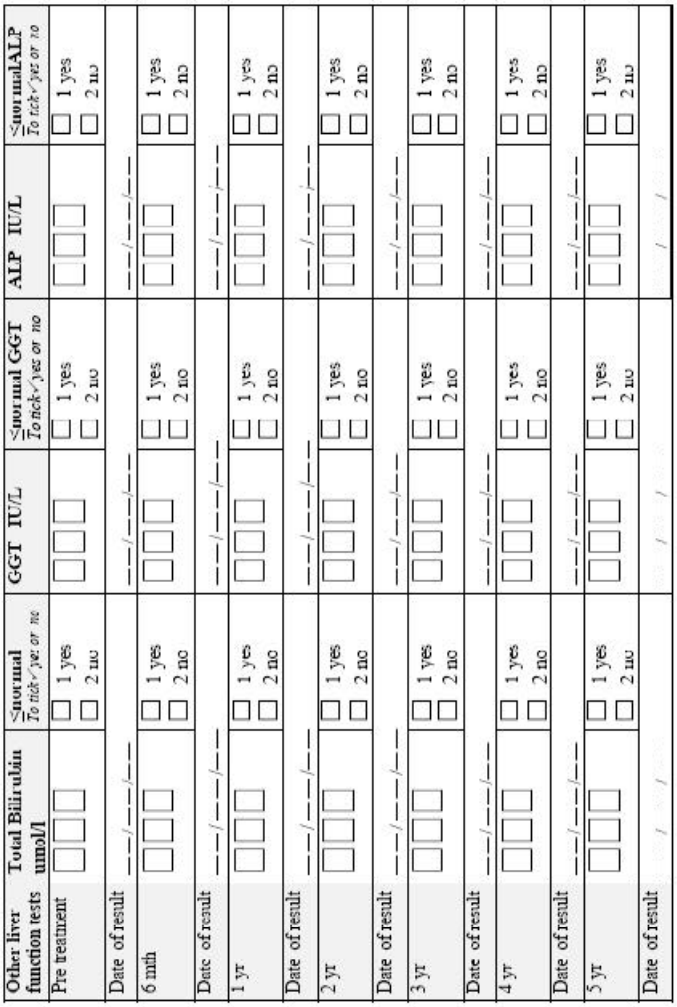

Other liver function tests	Total Bilirubin umol/l	≤normal *To tick yes or no*	GGT IU/L	≤normal GGT *To tick yes or no*	ALP IU/L	≤normalALP *To tick yes or no*
Pre treatment	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
6 mth	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
1 yr	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
2 yr	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
3 yr	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
4 yr	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	__/__/__		__/__/__		__/__/__	
5 yr	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no	☐☐☐	☐ 1 yes ☐ 2 no
Date of result	/ /		/ /		/ /	

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