CHAPTER I

INTRODUCTION

1. Background and Rationale

Hepatitis C Virus (HCV) has been identified as the major etiologic agent causing most cases of posttransfusion non-A, non-B hepatitis (NANBH) worldwide. Since the discovery of the HCV by molecular cloning in 1989 (1), more than one million new cases of infection are reported annually. Although primary infection with HCV is often asymptomatic, most individuals will develop a chronic infection that can lead to cirrhosis, decompensated liver disease and hepatocellular carcinoma (HCC) (2). During 1988-1994, the data from the Third National Health and Nutrition Examination Survey (NHANES III) have indicated that an estimated 3.9 million (1.8%) Americans have been infected with HCV (3). In Thailand, John Cohen reported that an estimated of 1-2% Thais have been infected with HCV (4). Most of these persons are chronically infection and might not be aware of their infection because they are not clinically ill. Although HCV infection occurs among persons of all ages, but the highest prevalence rates of HCV infection are found among persons aged 30-49 years and males predominate slightly (3). The numbers of deaths attributable to HCV-related chronic liver disease could increase substantially during the next 10-20 years as this group of infected persons reaches ages at which complications from chronic liver disease typically occur. The HCV infection is an important public health problem in the near future.

HCV isolations from around the world show substantial nucleotide sequence variability throughout the viral genome. Based on the identification of the genomic differences, HCV has been classified into 6 major genotypes and more than 100 subtypes (5). HCV genotypes are numbered in order to their discovery and more closely related such as 1a, 1b, 2a, and 2b. Some genotypes are endemic worldwide, while others restricted to

distinct geographical region (6). HCV genotype 1a and 1b are the most common in the United States and also in Europe (7). In Japan, genotype 1b is the most common (8). In Thailand, genotype 3a is the most common, followed by genotype 1b, genotype 6 group and 1a.The other genotypes are not found (9). Previous studies indicated that HCV genotypes are associated with the development of hepatocellular carcinoma and seem to play a role in predicting response to α-interferon therapy (10). Identification of HCV genotype in infected patients may be useful for prediction of the success rate and optimal duration of α-interferon therapy with or without ribavirin, and possibly for prediction of clinical progression to chronic infection. For example, the HCV genotype 2 and 3 present the better response than genotype 1a and 1b (11).

Since interferon therapy is expensive and may cause serious adverse effects, it would be useful to be able to predict the efficacy of interferon in HCV infection. It seems clear that the HCV genotype is very important and should be thoroughly investigated. The varieties of technologies have been examined for utility in the determination of HCV genotype in the clinical laboratory setting. However, the reference method for HCV genotyping is sequencing of a specific PCR-amplified portion of the HCV genome and followed by phylogenetic analysis (12). Recently, the HCV genotyping kit was commercial available. However, this kit was expensive. Therefore, the purpose of this study is to develop a HCV genotyping method by amplification and direct sequencing of 5' non coding region (NCR) of HCV genome. The result will be evaluated with the commercial kit. This method is considerably less expensive than currently available commercial kits and probably have more efficiency in HCV genotyping.

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2. Research Question

Major question

Can an in-house HCV genotyping method be developed by amplification and sequencing analysis of 5' NCR and follow by phylogenetic analysis with Clustal W program?

Minor question

Can the efficiency of genotyping increase the sequence length of 5'NCR from 183 bp to 264 or 332 bp, since the commercial kit can not differentiate some subtype such as 1a from 1b?

3. Limitation of the Study

This method depends on the Clustal W program and the input database of HCV references sequences which has to be updated when new references sequences or genotypes are available.

4. Objective of this Research

The aim of this study

1. To genotype the HCV from infected patients in King Chulalongkorn Memorial Hospital by sequencing of the 5' NCR and followed by phylogenetic analysis with Clustal W program.
2. To study the prevalence of HCV genotype in Thai patients from King Chulalongkorn Memorial Hospital.
3. To identify the difference portion of 5' NCR in each genotype which increases the efficiency of HCV genotyping.

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5. Hypothesis

1. Amplification and direct sequence of the 5' NCR followed by sequence comparison and phylogenetic tree construction can be used to determine the HCV genotype from infected patients.
2. The efficiency of this genotyping method can increase by the other regions in 5' NCR sequencing.

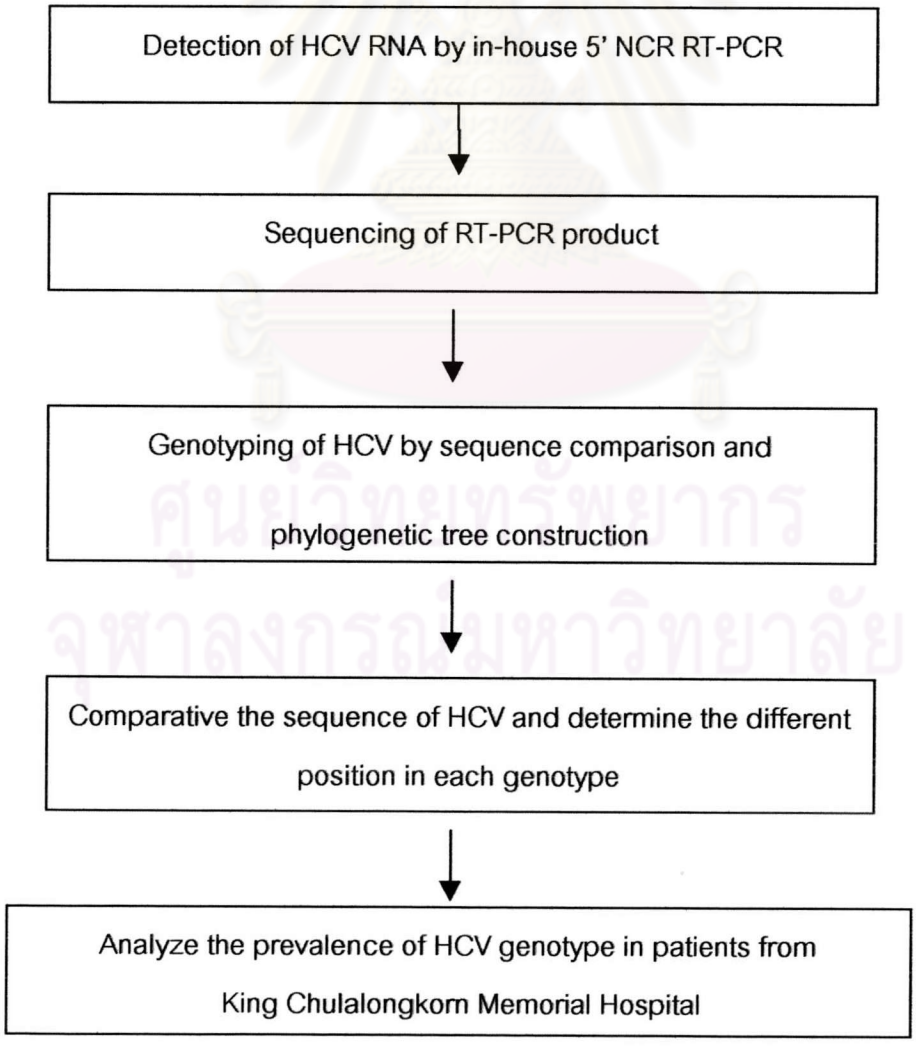
6. Key Words

Hepatitis C Virus (HCV)

Genotying

5' Non Coding Region (5' NCR)

7. Conceptual Framework



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8. Expected Benefits and Application

1. This method can be used to determine the HCV genotype for patients with low cost compare to commercial kit.
2. The prevalence of HCV in each genotype in patients from King Chulalongkorn Memorial Hospital is determined.
3. The extended portion of 5' NCR in each genotypes of HCV which increase the efficiency of HCV genotyping in this method is determined.
4. As the database for clinician in investigate the clinical significance of some HCV genotypes in liver diseases or response to therapy especially genotype which is in Thailand or Southeast Asia.

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