CHAPTER I

INTRODUCTION

Background and Rationale

Dengue is an endemic mosquito-borne viral disease affecting humans worldwide and constitute a major public health problem in tropical and subtropical regions (1, 2). Dengue viruses are grouped into four distinct serotypes, designated DEN-1, DEN-2, DEN-3, and DEN-4 (3, 4, 5). Dengue viruses were transmitted to man by the bite of a domestic mosquito, with *Aedes aegypti* being the principal vector (6, 7). The persons infected with dengue virus are generally clinically mild or asymptomatic, but may also present with undifferentiated fever, classical dengue (DF), dengue hemorrhagic fever (DHF) or dengue shock (DSS) (5, 8,9). The World Health Organization (WHO) categorizes DHF into four grades, from less severe (grade 1) to severe (grade 4). DHF grades 3 and 4 in which plasma leakage is so profound that shock occurs, are also referred to as dengue shock syndrome.

Dengue viruses are currently transmitted in more 100 countries. The World Health Organization (WHO) estimated that 50-100 million cases of dengue virus infections occur yearly, with 500,000 cases that required hospitalization and 15,000 deaths. Mortality rates vary from < 1%-10% (10).

The mechanism of DHF/DSS pathogenesis is still poorly understood. There are many theories explaining factor contributing to disease severity. These are immune enhancement, viral factors (virulence by genotypes or strains of dengue virus), host individual factors (genetic variation), and others (such as sickle cell anemia) (12, 13, 14, 15, 16, 17).

There exist multiple serotypes of dengue virus in *Aedes aegypti* and *Aedes albopictus* in Southern Thailand. This makes it possible for a human to get a

multiple infection with two or more different serotypes of dengue viruses from a single bite of the female mosquito infected with multiple serotypes of dengue virus (18)

There are many reports on clinical multiple dengue infections. In 1999, M. A. Lorono-Pino et al collected viremic serum samples and performed mosquito inoculation. These samples were collected during epidemics involving multiple dengue virus serotypes in Indonesia, Mexico, and Puerto Rico. 5.5% of the samples were found to contain 2 or more dengue viruses by an indirect immunofluorescence test and reverse transcription-polymerase chain reaction (19). In 2003, Wei Kung Wang, et al detected and determined the serotypes of 21 dengue patients during an outbreak in southern Taiwan in 2000 by multiplex reverse transcription polymerase chain reaction. 9.52% were concurrent infections by dengue type 2 and dengue type 3 virus (82). In the same year, Phaisan Khawsak et al investigated dengue virus serotypes of human blood samples in Thailand during 2000-2001 by RT-PCR technique. They found multiple infections with two or more dengue virus serotypes in 5% of the samples (21). In addition, in 2008, Preeti Bharaj et al studied about multiple infections of dengue virus in India, they found 19% with two dengue serotypes (22). The percentages of multiple infections seem to be increasingly documented in many countries.

In 2002, our laboratory was able to detect dengue infection by reverse transcription-nested polymerase chain reaction in urine specimens (23). Subsequently, our preliminary study of multiple infections with two or more dengue serotypes has identified this phenomenon in PBMC samples. We therefore speculated that nonblood samples such as saliva, urine, oral brush may be affected by such phenomenon as well.

The serotyping and genotyping study may help us to understand dengue virus epidemiology and pathogenesis in more details. Furthermore, this may be associated with manifestations of DHF/DSS. Different serotypes and genotypes of dengue in different blood and body-fluid compartments of individual patients may be associated with disease severity. We hypothesized that serotypes and genotypes of dengue virus may not always be consistent and uniform in blood and body fluid compartments in all patients. It is also interesting whether different serotypes and

genotypes could be associated with clinical outcomes, even though this is not a main objective of this study.

Research Questions

Research Question: Is there a difference in dengue serotypes and genotypes between blood and body fluid compartments of acutely-infected individual patients?

Objectives

1. To determine the difference of serotypes and genotypes in blood and body fluid compartments of acutely-infected individual
2. To determine the distribution of serotypes and genotypes in blood and body fluid compartments of acutely-infected individual

Hypothesis

Since viral strains may play an important role in the pathogenesis of dengue infection and disease severity, we sought to determine serotypes and genotypes of dengue virus in various blood and body fluid compartments. This conforms to the fact that RNA viruses generate "errors" during their replication. Differences in viral strains in blood and body fluid compartments may be associated with individual clinical outcomes as well as transmission efficiency of the virus.