CHAPTER I

INTRODUCTION

Background and rationale

Dengue virus (DENV) infection becomes a major health problem in many tropical and subtropical countries. There are approximately 50-100 million people suffering from this infection every year. In Thailand, the prevalence of DENV infection is reported in all regions and a number of infected patients increase every years. The age of infected patients is not limited in children but also found in adolescences and adults. DENV is single-strand positive-sense RNA virus belonging to family *Flaviviridae* containing approximately 10,700 bases in length and divided into four serotypes (DENV1-DENV4). The genome organization composes of 3 structural and 7 non-structural genes. In structural genes, there are capsid (C), pre-membrane (prM) and envelope (E) genes, whereas non-structural (NS) genes are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. DENV is an arthropod-borne virus transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes [1-3]. After infected with DENV, more than 90% of infected patients are suffered from asymptomatic infection, but only 10% with heterotypic infection are possible to develop symptomatic appearance ranging from undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) to dengue shock syndrome (DSS). Antibodies against one serotype of DENV cannot protect others; furthermore, it causes severe infection resulting from antibody-dependent enhancement (ADE) mechanism after infected with different serotypes during secondary infection [4]. Generally, DENV infection is a self-limited disease. Viral particles are neutralized and eliminated by the effect of neutralizing antibodies. In other flaviviruses such as hepatitis C virus (HCV), West Nile virus (WNV) and St. Louis encephalitis virus (SLEV), the evidence of persistent infection are described [6-11]. For example, Tonry *et al.* demonstrated the presence of WNV in the urine of infected hamsters up to 2 months

[10]. Murray *et al.* supposed that WNV could be detected in convalescent urine of acute and chronic WNV-infected patients during 1.6 to 6.7 years after infection [7]. According to DENV, there are a few studies describing prolonged detection of DENV in urine and plasma as late as day 16 and day 11 of illness, respectively [12, 13].

DENV infection is a systemic infection similar to other DNA and RNA viruses. It commonly replicates in white blood cells (PBMCs) and dendritic cells, then viral particles spread via blood stream called "viremia" into many organs such as liver, kidneys, neuron cells and spleen [14, 15]. In addition, DENV particles are secreted into many body fluids, especially in urine known as "viruria" and in saliva of infected patients akin to other viruses such as human immunodeficiency virus (HIV), adenovirus and WNV [16-18]. Due to the multi organ infections of DENV, viral loads are different among body compartments. Additionally, the viral loads in different time points of each compartment were also different. For example, Poloni *et al.* showed the different viral loads of DENV (PFU/ml) by real time RT-PCR (qRT-PCR) among serum, saliva and urine during acute period (day 2 of illness)[19]. However, the limitation of previous study is the small sample size and does not compare the viral load among different time points.

RNA viruses normally present the high rate of genetic variation. There are many types of variation such as mutation, recombination and reassortment, which is the effect of RNA-dependent RNA polymerase (RdRp) activity creating some errors during each replication because of the lacking proof-reading activity. As a result, RNA viruses are composed of heterogeneous populations known as "quasispecies" [20, 21]. This term means "the closer but non-identical mutant and recombinant viral genomes in the same population" occuring when viruses adapt themselves to a new environment such as in a new host or a target site in which they infected and when the viruses develop an escape mechanism from host immune response [21]. Quasispecies may also front to the selection of virulent strains and the evolution of new viruses, which contributes to the new pathogenesis of viral infection and affects the stimulation of long-term survival or persistent infection.

Several studies suggest that DENV plays a role in genetic variation or quasispecies during each infection [22-26]. The consequential outcomes of this phenomenon correlate with pathogenesis of DENV infection. Genetic variations are

described in both structural and non-structural genes, especially in domain III of E gene. This gene consists of approximately 1,485 nucleotides and is translated to E protein (~495 amino acids), which has many biological functions involving in pathogenesis of infection such as a receptor binding protein and a neutralizing antibody epitope [25, 27]. The variation of E gene relates with viral adaptation and selection as well as virulence of infection. Additionally, C, NS1 and NS2B genes were studied as well [25, 26]. Previous studies in DENV interested to explore in acute specimens in a single period of infection. Nevertheless, there has been no study describing the genetic variation of DENV in both different specimens and time points.

According to previous studies in our laboratory, DENV was detected in various body compartments such as in plasma, PBMCs, saliva and urine during acute period [28, 75-77]. Moreover, DENV was cultured from urine by using a mosquito inoculation technique up to 28 days after onset of illness [29]. Interestingly, our previous project, "Survival of dengue virus in blood, urine, saliva and buccal mucosa in complete-recovery dengue patients", we found that DENV could be detected for a long period in PBMCs and urine [30]. However, confirmation studies were required to prove these findings. Moreover, there was no study to describe more details of our previous results such as the longest time of detection, the viral load and the genetic variation in different time points. The purpose of this study was to investigate more details about the long-term DENV infection by monitoring the longest time of detection, studying the viral load and exploring the genetic variation in different body compartments (specimens) and time points. In this study, we focused on adult patients because the rate of infected patients drastically increases every year and there are few studies about adult patients in Thailand. Our findings may clearly describe more details about pathogenesis of DENV and be useful for DENV surveillance and vaccine development.

Objectives

1. To monitor the longest time that DENV can be detected and which specimen is suitable for persistent DENV in adult patients.
2. To compare the serotypes, genotypes and strains of DENV in different time points of DENV-infected adult patients.
3. To study the viral load and genetic variation of DENV in different time points of DENV-infected adult patients.
4. To monitor whether detected DENV in each specimen in different time points is alive.

Definitions

Monitoring of DENV infection

We monitor the DENV and non-DENV-infected adult patients beginning from day 1 at admitted or day 1 of illness until day 90 of illness. It is divided in to acute, early convalescent and late convalescent periods, which corresponds to our arbitrary criteria and clinical symptoms of each patient.

Acute or febrile period

It means the period that DENV and non-DENV-infected adult patients get fever.

Early convalescent period

This period begins from the first day of fever recovery until day 25 of illness.

Late convalescent period

This period begins from day 26 of illness but not later than day 90 of illness.

Blood specimens

It means plasma and PBMCs.

Non-blood specimens

It means saliva and urine.

Key words

Dengue virus, genetic variations, quasispecies, viral load, urine, saliva

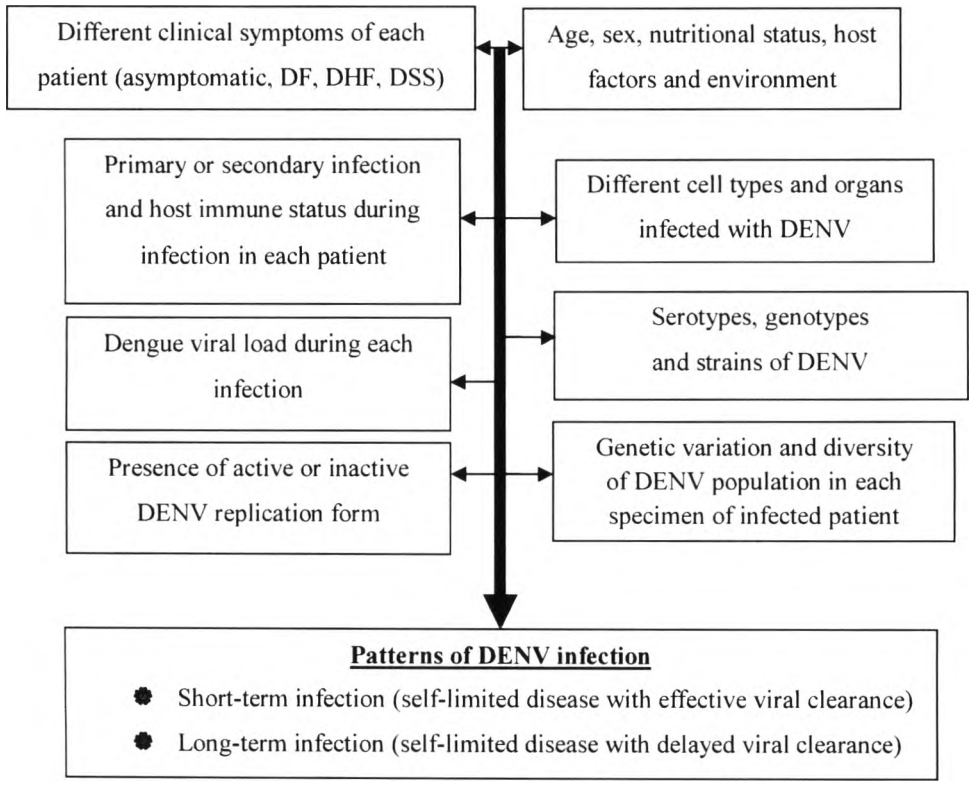
Major research question

How long can DENV be detected in DENV-infected patients and how are the serotypes, genotypes and strains as well as genetic variations in various time points of DENV-infected adult patients?

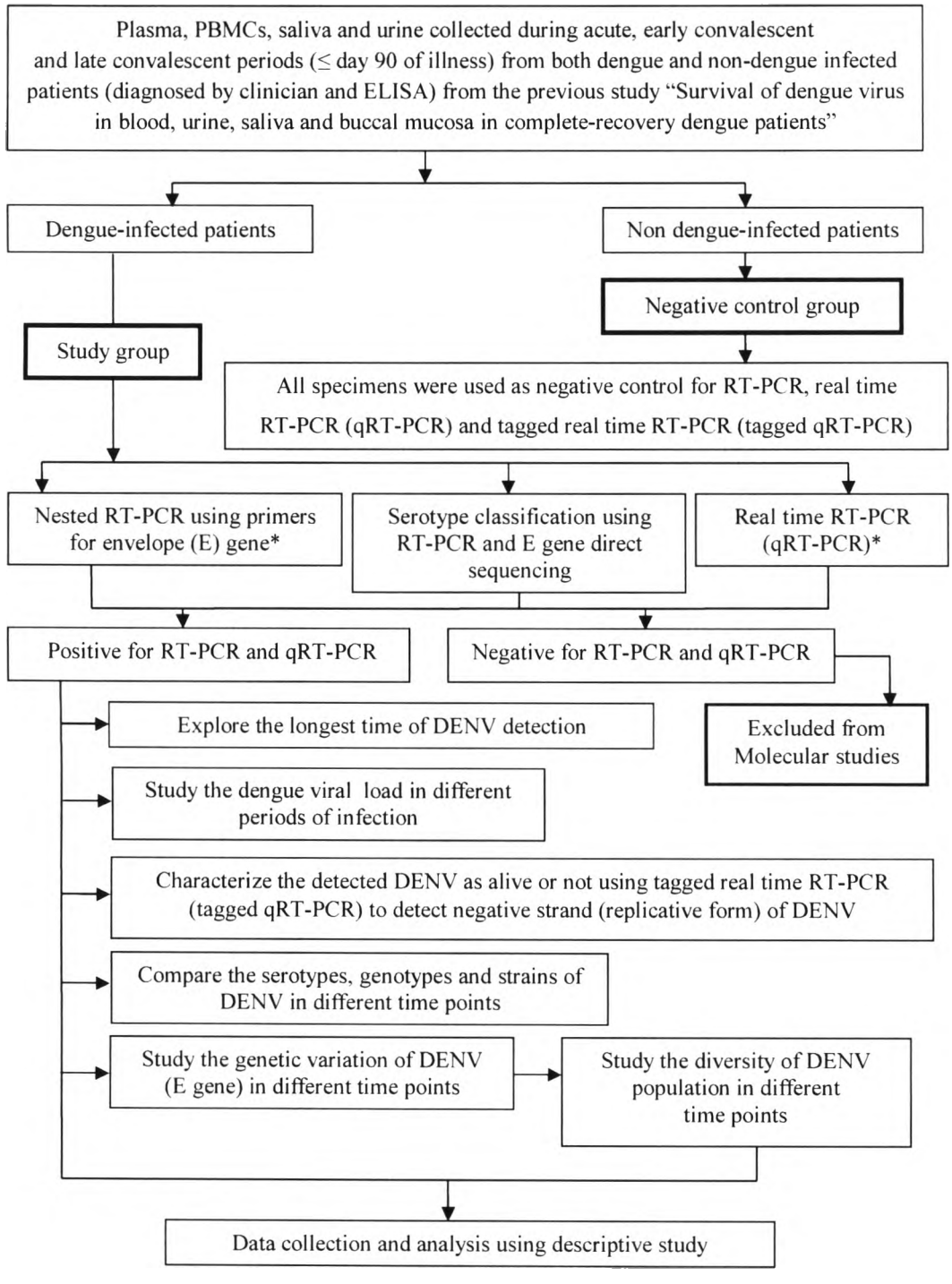
Minor research questions

1. How do the viral load of DENV present in different time points of DENV-infected adult patients?
2. Is the active form of DENV detected in positive specimen for DENV in different time points?

Conceptual framework



Experimental design



* Used as major methods for monitoring the longest time of DENV detection.

Research design

Descriptive study (prospective study)

Expected Benefits and Applications

Our findings will describe more details about pathogenesis of DENV infection in case of the persistent infection, serotypes, genotypes and strains of DENV in different body compartments and time points. We will also illustrate the genetic variation, the complexity of DENV population, the viral load and the presence of replicative form of DENV in different periods of infection. These points may help some scientists to study about viral surveillance, to develop effective vaccines and to understand pathogenesis of DENV infection.

Limitations

1. Lost-to-follow-up patients may affect the sample size in this study.
2. Specimens collected at weekend which could not be transferred to process in laboratory immediately and preserved specimens may decrease the efficiency of viral detections because of RNA degradation.

Ethical consideration

This project was approved by Institutional Review Board (IRB) of Ethics Committee, Faculty of Medicine, Chulalongkorn University, Thailand dated October 20, 2009 (COA No. 877/2009).