CHAPTER I

INTRODUCTION

The importance of HCMV detection

HCMV infection in the general population

Human cytomegalovirus (HCMV) is found universally throughout all geographic locations and socioeconomic groups. The prevalence of the HCMV antibody in adults ranges from 30-97%[1,2]. HCMV is the most common congenital viral infection in humans, due to its high prevalence in the general population, with up to 90% of the urban population infected. HCMV is spread from mother to baby by three routes: transplacental, intrapartum, and human milk (Table 1). Transplacental infections occur in women who were infected long before conception (recurrent infection) as well as in those infected with primary HCMV during pregnancy. Intrauterine transmission of HCMV occurs in approximately 40%[1] of pregnant women with primary infection; an estimated 1:100 infants are congenitally infected. The incidence of primary HCMV infection in pregnant women in developed countries and Thailand were reported to be 0.4-7%, and 8.6%, respectively[2]. However, during pregnancy, prior immunity to HCMV in the mother can reduce the viral inoculum transmitted to the fetus, resulting in a reduced risk of neonatal disease[3,4]. Young children in day-care centers have been shown to transmit the virus to other children and susceptible adults. In populations from a high socioeconomic environment, approximately 40% of adolescents are seropositive; thereafter, the rate of virus acquisition increases by approximately 1% per year[3]. HCMV infection can be sexually transmitted. HCMV seroprevalence is also high among homosexual males and patients examined at sexually transmitted disease clinics. Primary infection commonly occurs by direct close personal contact – exposure to bodily secretions such as saliva, tears, urine, stool, semen, and breast milk. After primary infection, HCMV remains latent in tissue and peripheral blood neutrophils, monocytes, and endothelial cells, and intermittent shedding that results from reactivation commonly occurs

without any marked disease consequences. Some persons with symptoms experience mononucleosis–like a syndrome with prolonged fever, and mild hepatitis.

Table 1 Vertical transmission of human CMV infection[4]

Route	Maternal situation	Rate of CMV infection in infant
Transplacental	Seropositive before conception	0.2%-2.2%
	Primary gestational infection	20%-40%
Intrapartum	Seropositive	5%
	Virus in genital tract at term	50%
Breast milk	Seropositive mother	25%
	Virolactia	65%-70%

HCMV infection in immunocompromised patients

In AIDS patients with a CD4 T-cell count of below 50 cells/mm^3, HCMV is a leading cause of retinitis (85.3%), esophagitis (9.2%), colitis (7.3%) and both retinitis and esophagitis (2.7%).

In transplant recipients, three major patterns of HCMV transmission are observed. Primary infection occurs when a HCMV-seronegative individual receives organ(s) from a seropositive donor, followed by reactivation of the latent virus. Secondary or reactivation infection occurs when the endogenously latent virus is reactivated posttransplantation in a seropositive recipient. Superinfection or reinfection occurs when the latent virus of donor origin is reactivated following transplantation to a HCMV-seropositive recipient. Primary infection acquired from the transplanted organ is generally more severe than reactivation, although this varies with specific organs and the degree of immunosuppression. The rates of HCMV infection and disease in kidney/liver transplant patients, according to type of infection, are summarized in Table 2. In bone marrow transplantation, pneumonitis is the most common clinically significant HCMV disease. For allogeneic transplants, it has been reported that 0% to 15% of seronegative recipients receiving marrow from a positive donor, and 6% to 36% of seropositive patients

receiving marrow from a positive donor have HCMV pneumonia. In contrast, HCMV pneumonia has been reported in 2.1% to 8.5% of autologous marrow transplant patients[2, 4].

Table 2 Rates of HCMV infection and disease in kidney and liver transplant patients, according to type of infection[4]

Pretransplant Serostatus of Donor/recipient	Type of infection	Kidney		Liver	
		Infection (%)	Disease (%)	Infection (%)	Disease (%)
+/+	Reactivation or reinfection	51	19	66	23
+/-	Primary	67	50	77	61
-/+	Reactivation	59	12	49	10
-/-	Primary, not from graft	0	0	10	10

The importance of laboratory diagnosis

HCMV infection is responsible for significant morbidity and mortality in at least 2 groups of patients, AIDS patients and organ transplant recipients. In Thailand, AIDS has become the leading cause of death and an estimation of more than one million people are infected with HIV. The HCMV reactivation in transplant recipients, who receive steroid or immuno-suppressive compounds to prevent graft rejection, is needed to be carefully monitored. Until now, the serologic tests for specific immunoglobulin G and M antibodies are the major methods used to detect HCMV infection in Thailand. However, this technique was found to be low in sensitivity and is not reliable as a marker of HCMV infection in organ transplant patients[5]. Thus, the polymerase chain reaction (PCR)-based methods are suggested in order to increase the efficiency of HCMV diagnosis. There are commercial HCMV DNA/mRNA detection kits already available, but they have the limitation of high cost at approximately 800-2,000 Baht/test.

Therefore, the researcher is interested in establishing an in-house nested PCR technique to provide a cheaper, sensitive and specific method for the detection of HCMV DNA. Regarding this, optimization of PCR conditions was performed. The HCMV DNA control was constructed and used as a positive control, whereas an internal control was also used to maximize

the validity of the test. The optimized PCR conditions were evaluated by performing a diagnosis of HCMV retinitis and monitoring the HCMV reactivation in kidney transplantation patients.

The objectives of this study;

1. To optimize the duplex nested PCR conditions for HCMV DNA detection.
2. To evaluate the sensitivity and specificity of the optimized duplex nested PCR for the detection of HCMV DNA.
3. To compare the efficiency of HCMV detected in different ocular samples from retinitis patients.
4. To compare the specificity and sensitivity of HCMV diagnosis between the optimized duplex nested PCR and conventional nested PCR in retinitis patients, and between the optimized duplex nested PCR and anti-HCMV IgM antibody in post-transplant recipients.