UNIVERSITI PUTRA MALAYSIA

ASSOCIATION BETWEEN HUMAN CYTOMEGALOVIRUS RELATED FACTORS AND DEVELOPMENT OF THE DISEASE IN RENAL AND BONE MARROW TRANSPLANT RECIPIENTS IN A TERTIARY HOSPITAL, MALAYSIA

MOHD FAHMI BIN MASTUKI

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MOHD FAHMI BIN MASTUKI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science

May 2014

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Dedicated with love and gratitude to:

Supervisor:
Dr. Niazlin Mohd Taib

Father:
Mastuki bin Damar

Mother:
Laili binti Gupar

Brother and Sisters:
Mohd Azhari, Mohd Azizi, Mohd Fahrul Razi, Mohd Farid, Mohd Hafizuddin, Norhafizah, Mohd Faidz.

Wife and Children:
Siti Fairuz Abdul Rashid and Muhammad Hanif bin Mohd Fahmi and Naurah Hani binti Mohd Fahmi.

“The love of a family is life’s greatest blessing”

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ASSOCIATION BETWEEN HUMAN CYTOMEGALOVIRUS RELATED FACTORS AND DEVELOPMENT OF THE DISEASE IN RENAL AND BONE MARROW TRANSPLANT RECIPIENTS IN A TERTIARY HOSPITAL, MALAYSIA

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May 2014

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Human cytomegalovirus (HCMV) infection is known to be a major infectious complication after transplantation which associated with significant morbidity and mortality in solid organ and bone marrow transplant recipients. We studied the viral factors of HCMV and correlate results with the development of HCMV disease. This aim of this study is to detect HCMV, their genotypes and co-infection with other herpesviruses namely Epstein-Barr virus (EBV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7) in post-solid organ and bone marrow transplant recipients and to correlate them with the clinical presentation and outcome of HCMV disease. In this study, 100 blood samples from renal transplant recipients and 100 bone marrow transplant recipients in Kuala Lumpur Hospital were included. All tests were carried out by real time polymerase chain reaction (qPCR). HCMV were detected in higher incidence compared to other herpes virus indicating that the virus was the most common virus infecting the immunosuppressed patients. The results revealed that the incidence of HCMV infection were 78% and 63% in renal and bone marrow transplant recipients respectively. We found that patients with high viral load show symptoms of HCMV disease, whereby fever being most common symptom. As Malaysia has multi-races citizens, we also demonstrate the incidence of HCMV infection among renal and bone marrow transplant recipients by ethnicity namely Malay, Chinese, Indian and other minority races as ‘others’. In renal transplant recipients, there was no significant difference between the ethnic. Nevertheless, we found that there was a significant HCMV positivity among races in bone marrow transplant recipients where Malays were the most infected. One of the pathogenesis of HCMV depends on the genes encoding envelope glycoprotein that associated with different clinical outcomes. Reactivation of latent viral infection by HCMV and other herpesviruses results in active viral infection after organ transplantation and may cause complications. HCMV genotyping analysis revealed that all three HCMV gB, gH and gN genotypes were presence in the population where gB1 strain being the most common gene detected in both renal (100%) and bone marrow (100%) transplant recipients. Mix infection by more than one HCMV genotypes was also detected with various percentages with the gB+gH+gN combination was the least type of

mix infection. We also found that recipient with high HCMV viral load (>5,000 copies/mL) has increased risk of developing HCMV disease. No statistically significant difference was found between type of genotypes and the manifestation of HCMV disease ($p>0.05$). Co-infection with other herpesviruses with HCMV disease was significant in bone marrow transplant recipients but not significant in renal transplant recipient.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNGKAIT ANTARA FAKTOR-FAKTOR BERKAITAN SITOMEGALOVIRUS MANUSIA DENGAN PEMBENTUKAN PENYAKITNYA DIKALANGAN PENERIMA ORGAN BUAH PINGGANG DAN SUM-SUM TULANG DI SEBUAH HOSPITAL TERTIARY, MALAYSIA

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Jangkitan sitomegalovirus manusia (HCMV) telah diketahui sebagai komplikasi jangkitan yang utama yang berlaku selepas pemindahan organ dimana ia dikaitkan dengan kadar morbiditi (kejadian) dan mortaliti (kematian) di kalangan penerima pemindahan organ dan sumsum tulang. Kami mengkaji faktor-faktor virus HCMV dan menghubungkaitkan dengan penyakit HCMV. Tujuan kajian ini dijalankan adalah untuk mengesan HCMV, genotip-genotipnya, dan jangkitan bersama dengan herpesvirus-herpesvirus yang lain iaitu virus Epstein-Barr (EBV), herpesvirus manusia jenis 6 (HHV-6) dan herpesvirus manusia jenis 7 (HHV-7) di kalangan penerima organ dan sumsum tulang dan mengaitkan faktor-faktor ini dengan tanda-tanda klinikal dan akibat oleh penyakit HCMV. Sebanyak 100 sampel darah dari penerima buah pinggang dan 100 sampel darah dari penerima sumsum tulang daripada penerima-penerima organ di Hospital Kuala Lumpur telah dimasukkan di dalam kajian ini. Semua ujian dijalankan dengan menggunakan teknik *real time polymerase reaction* (qPCR). HCMV dikesan pada kadar yang lebih tinggi berbanding dengan herpesvirus lain. Ini menunjukkan bahawa ia adalah virus yang biasa menjangkiti pesakit-pesakit berimuniti rendah. Dalam populasi kajian ini sebanyak 78% dan 63% jangkitan HCMV masing-masing terhadap penerima buah pinggang dan sumsum tulang. Kami mendapati bahawa pesakit yang mempunyai jumlah *viral load* yang tinggi menunjukkan gejala-gejala HCMV dimana demam menjadi gejala yang paling banyak didapati. Oleh kerana Malaysia mempunyai pelbagai kaum iaitu Melayu, Cina, India dan lain-lain, kami mengkaji kadar jangkita HCMV dikalangan etnik tetapi tiada perbezaan yang ketara secara statistik yang tentang jangkitan HCMV dengan jenis etnik. Walaubagaimanapun, kami mendapati kadar jangkitan HCMV yang ketara di kalangan pesakit yang menerima sumsum tulang bangsa Melayu. Salah satu pathogenesis HCMV adalah bergantung kepada gen-gen yang mengawalatur sampul *glycoprotein* yang dikaitkan dengan pelbagai konsekuensi klinikal. Pengaktifan semula jangkitan virus latent oleh HCMV dan herpesvirus-herpesvirus yang lain mengakibatkan jangkitan virus aktif selepas pemindahan organ dan boleh menyebabkan komplikasi. Analisis genotip HCMV menunjukkan bahawa ketiga-tiga genotip hadir didalam populasi kajian ini dimana jenis gB1 adalah gen yang paling banyak dikesan didalam kedua-

dua jenis pemindahan organ iaitu 100% setiap jenis. Jangkitan campuran oleh lebih daripada satu genotip HCMV juga dikenalpasti dengan peratusan yang berbeza-beza dimana kombinasi jangkitan gB+gH+gN menjadi kombinasi yang paling sedikit menyebabkan jangkitan campuran Kami juga mendapati bahawa penerima organ yang mepunyai viral load HCMV yang tinggi (>5,000 *copies*/mL) meningkatkan risiko untuk mempunyai penyakit HCMV. Tiada perbezaan statistik yang jelas didapati antara jenis-jenis genotip dengan manifestasi penyakit HCMV. Kaitan jangkitan bersama antara herpesvirus-herpesvirus yang lain dengan penyakit HCMV didapati signifikan di kalangan penerima sumsum tulang ($p<0.05$) manakala tidak signifikan di kalangan penerima organ buah pinggang.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	Degree Celcius
µL	Micro Liter
µM	Micro Moles
cop/µL	Copies per Micro Liter
DNA	Deoxyribonucleic Acid
L	Liter
mL	Milliliter
NC	Negative Control
PC	Positive Control
IC	Internal Control
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
HCMV	Human Cytomegalovirus
HHV-6	Human Herpervirus- Type 6
HHV-7	Human Herpesvirus- Type 7
EBV	Epstein Barr virus

CHAPTER 1

INTRODUCTION

Organ transplantation such as liver, kidney, heart, lung and bone marrow is a well-known therapeutic option for many human diseases. It has become a standard therapy for selected end-stage diseases such as renal and bone marrow disease (Cukuranovic *et. al.,* 2012). However, complications may arise after transplantation which includes infection, allograft rejection and side effects of immunosuppressive therapy which remain major causes of morbidity and mortality following solid organ transplantation (Cukuranovic *et. al.,* 2012). According to Kotton & Fishman (2005), viruses are the most common cause of opportunistic infection in post transplantation which can leads to several complications.

Many viral infections after renal and bone marrow transplantation result from reactivation of “latent” viral infection in the host or from the graft. Latency means the virus exist in the host after primary infection then remain in inactive stage. Some of the virus “awakes” due to some reasons including the nature of the virus, host immune response and infection of tissues (Kotton & Fishman, 2005). While other viruses are constantly replicating at low levels, some latent viruses are metabolically inactive, determined by the effectiveness of the hosts immune response. Few factors contribute to viral activation after transplantation, including immune suppression (especially reduction of cytotoxic immunity), graft rejection therapy, inflammation (cytokines), and tissue injury (Kotton & Fishman, 2005; Cukuranovic *et. al.,* 2012).

The risk of infection in transplant recipients is determined by the intensity of exposure to potential viruses (epidemiologic exposure) and factors that cause patient susceptible to infection (immunosuppression) (Fishman & Rubin, 1998). Some incidence of viral infections result from community exposure such as influenza and adenovirus, while some are commonly transmitted within the allograft such as human cytomegalovirus (HCMV) and Epstein-Barr virus, and other are reactivated in the setting of immunosuppression such as varicella zoster virus (Kotton & Fishman, 2005). Moreover, the use of immunosuppressive agents to prevent the rejection of transplanted organs has increased the patients’ susceptibility to opportunistic infection (Fishman, 2007).

Neonatal and immunocompromised patients usually associated with a wide range of diseases caused by HCMV infection (Xia & Zhang, 2010). HCMV infections can cause morbidity and mortality in neonatal and immunocompromised patients although advanced antiviral therapy has been introduced after renal and bone marrow transplantation to prevent the disease. It usually happened because of mutations associated with antiviral resistance (Xia & Zhang, 2010). Besides that, combination of viral factors and host immune responses also contribute to the pathogenesis of HCMV. Genomic polymorphism is one of the viral factors that usually associated with different clinical outcomes. Various genomic polymorphism that have been demonstrated among clinical isolates in the HCMV genes encoding envelope glycoprotein includes glycoprotein B (gB), glycoprotein N (gN), glycoprotein O (gO), glycoprotein H (gH), and glycoprotein L (gL) (Xia & Zhang, 2010).

Besides in transplantation and hospitalised patients, the seroprevalence of HCMV is currently demonstrated all over the world, which also presence in the community, both in under developing and developing countries. Prevalence of HCMV disease varies from 80 to 100% in Africa and Latin America whereas lower in the northern hemisphere countries, which were 40 to 60% (Thomasini *et al.,* 2012). In the Malaysian states of Selangor and Wilayah Persekutuan 92% seroprevalence of HCMV were reported from blinded study performed in 2012 among healthy blood donors (Camalxaman *et al*., 2012). In other studies done in Hospital Universiti Kebangsaan Malaysia (Jamal *et. al.,* 1998) and Hospital Universiti Sains Malaysia (Ahmad *et. al.,* 2006), it has been documented that both transfused thalassemic patients and regular blood donors have equally high seroprevalence rates. Thus, HCMV infection is expansive, universal and relevant to be studied intensely (Camalxaman *et al.,* 2012).

In solid organ transplant (SOT) recipients, HCMV has become a preventable cause of mortality and morbidity. If prevention strategy is not taken HCMV disease usually occurs during the first 3 months after SOT. However, this onset has been delayed in patients who received HCMV prophylaxis (Razonable & Humar, 2013). Due to aberrant immune response within the allograft, HCMV has the affinity to invade the allograft. Not only that, due to its ability to modulate the immune system, it also has numerous indirect effects. Bacteraemia, invasive fungal and Epstein–Barr virus-associated post-transplant lymphoproliferative disease are the types of infection that usually related to HCMV. Chronic allograft nephropathy (or tubulointerstitial fibrosis in kidney recipients), bronchiolitis obliterans (lung recipients) and coronary vasculopathy (heart recipients) are the types of acute and chronic allograft injury contributed by HCMV infection (Razonable & Humar, 2013).

This study is carried out to identify the HCMV genotype populations that present in the blood sample of transplant recipients. The different genotype populations of HCMV which are glycoprotein B, N and H in bone marrow and renal transplant recipients were identified by using the real-time polymerase chain reaction technique. In addition, this study also identify whether there are co-infection with multiple strains in HCMV infected organ transplant recipients. The identification of HCMV genotyping is very important in order to indicate various HCMV strains presence in transplant recipients and improve the therapeutic interventions. Although HCMV is the most common opportunistic pathogen seen in transplant recipients, many other viruses have also affected outcomes (Weikert & Blumberg, 2008). Co-infection with other herpesviruses, such as Epstein-Barr virus (EBV) human herpesvirus-6 (HHV-6) and human herpesvirus-7 (HHV-7) has been implicated as risk factors for progression from active HCMV infection to HCMV disease. Thus the presence of these viruses need to be identified in the same study population in order to elucidate whether their presence impose a threat on the transplant recipients.

Finally, we analysed the correlation between HCMV viral load, patient's demographic data, different HCMV genotypes, co-infection with other herpesviruses (HHV-6, 7 and EBV) as well as their contribution in the development of HCMV diseases in both the renal and bone marrow transplant recipients in Malaysia.

1.1 Problem Statement

Human cytomegalovirus (HCMV) often associated with a wide range of diseases, particularly in transplant recipients undergoing immunosuppressive therapy. Little is known about the clinical manifestation associated with HCMV viral load, specific viral genotypes and host factors among renal and bone marrow transplant recipients especially in Malaysia where data about HCMV is still lacking

1.2 Significance of Research

Solid organ and bone marrow transplant has increased worldwide since there was an improvement of immunosuppressive agents and graft survival. However, the administration of immunosuppressive drugs to prevent graft rejection cause the patient immune system becomes suppressed, thus resulting in an increased incidence of viral infection of post-organ transplantation. Viral infection such as human cytomegalovirus (HCMV) is associated with particular syndromes and morbidity in the immunocompromised patients.

1.3 Objectives of Research

1.3.1 General Objective

The aim of this study is to identify the correlation between HCMV viral load, HCMV genotypes, EBV, HHV-6 and HHV-7 DNAemia and HCMV disease after renal and bone marrow transplantation.

1.3.2 Specific Objectives

1) To determine the percentage of human cytomegalovirus DNA in renal and bone marrow transplant recipients by quantitative real time PCR.

2) To determine the genotypic distribution of HCMV envelope glycoprotein B, N and H in renal and bone marrow transplant recipients by using SYBR Green-based real time PCR.

3) To identify the presence of human herpesvirus type-6 (HHV-6), human herpesvirus type-7 (HHV-7) and Epstein-Barr virus (EBV) by using SYBR Green-based real time PCR.

4) To determine the correlation between HCMV viral loads, the occurrence of various HCMV glycoproteins, co-infection with EBV, HHV-6 and HHV-7 and development of HCMV disease in the same study population by statistical analysis.

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