ORIGINAL ARTICLE

Human Cytomegalovirus: A culprit of infection in renal and bone marrow transplant recipients in Malaysia

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Abstract:

Human cytomegalovirus (HCMV) infection may cause substantial morbidity and mortality after renal and bone marrow transplantation. The aims of this study were to determine the incidence of HCMV infection in renal and bone marrow transplant recipients and to investigate its associations with HCMV disease, gender and races. This analysis involved 200 blood samples from renal and bone marrow transplant recipients from January 2011 to June 2012. The overall incidence of HCMV infection in the study group was 70.5% (141/200) where renal and bone marrow transplants account for 78.0% (78/100) and 63.0% (63/100), respectively. The incidence of HCMV infection in renal transplantation differed significantly by sex ($p<0.05$) where it was higher in males (78.2%) than in females (21.8%) but there was no statistically significant difference by sex in bone marrow transplantation in which males and females account for 47.6% and 52.4% respectively. The incidence of HCMV infection was not significantly different ($p>0.05$) by races in both transplantation types. The percentages are as follows: 45% (Malay), 24% (Chinese), 17% (Indian) and 15% (other indigenous races) in renal transplantation while 75% (Malay), 3% (Chinese), 8% (Indian) and 13% (other indigenous races) in bone marrow transplantation. The most seen symptoms were fever followed by generalised lethargy and headache. This study has shown that HCMV viral load has no significant association between age, gender, races and HCMV disease. Treatment with anti-HCMV therapy results in decline in HCMV load, usually to undetectable.

Keywords: Bone marrow transplantation; HCMV disease; Human cytomegalovirus (HCMV) infection; renal transplantation; viral load

1. INTRODUCTION

Human cytomegalovirus (HCMV) is a beta-herpesvirus and belongs to the family of Herpesviridae. The virus spreads via excretion in nearly all body fluids, such as urine, saliva, vaginal secretions, semen or breast milk and is ubiquitously distributed among human population [1]. Several seroprevalence studies show that HCMV in adults ranges from 55% [2] in developed countries to as high as over 90% [3, 4] in developing countries. HCMV usually causes a mild latent infection in immunocompetent persons but it poses a significant health threat to immunocompromised individuals, such as in allograft organ transplant recipients which can lead to increased risk of graft loss. Despite significant advances in the field of transplantation, HCMV remains the main cause of morbidity and mortality in both solid organ transplant and bone marrow transplant recipients [2].

Malaysia is a multiracial country with a population of more than 30 million which consists of Malay, Chinese and Indian as many other indigenous races. Seroposivity identification of HCMV among the general population and blood donors indicate that 91% to 97.6% of Malaysian were infected with HCMV [5, 6]. However the prevalence of active HCMV in transplant recipients as well as in the graft donors has not being published before in Malaysia. As the management of transplant recipients has improved, an increased incidence and range of opportunistic infections particularly caused by HCMV is observed [7, 8].

In general population, exposure to the virus as indicated by the presence of detectable IgG anti-CMV antibodies in the plasma is increases with age [9]. This condition also exists in more than two-thirds of donors and recipients prior to transplantation [9] and therefore it is common for the donors and/or recipients to be HCMV-positive at the time of transplantation. In determining HCMV infection *in vitro*, polymerase chain reaction (PCR) has been proved to be a

sensitive and effective technique. Nowadays, quantitative real-time PCR (qRT-PCR) is widely applied for its preponderance in quantifying the viral load in order to identify and monitor the HCMV disease progression before and during pre-emptive and prophylactic therapy [10]. Thus, this study aims to determine the incidence of HCMV infection in renal and bone marrow transplant recipients.

2. MATERIALS AND METHODS

2.1 Patients

The patients' database were retrospectively reviewed comprising of 100 renal and 100 bone marrow transplant recipients in which their samples were sent for HCMV DNA testing. The samples were routinely monitored on a weekly basis for a 3-month period after transplantation. Follow-up of the patient were extended up to one year to monitor the HCMV-associated disease manifestation. However, due to difficulty in getting consecutive specimens, we analyzed one sample per patient who had been transplanted for less than 1 year period. Data on the prescribed antiviral therapy were not completely obtained for all patients. Ten percent of renal transplant recipients with high HCMV load were recorded to receive ganciclovir, val-ganciclovir or acyclovir as prophylaxis therapy. Pre-emptive therapy was used in all bone marrow transplant recipients. However, 3% were recorded to receive antiviral drugs again due to high HCMV load. The study was approved by the National Medical Research Register review board (ID: NMRR-11-1124-10155). Samples received in the virology laboratory were serum, blood or plasma. Patient information such as demographics, indications for transplantation, symptoms appeared, sample types and the condition of the sample received were obtained together with the samples. However the serostatus of the donor and the recipients could not be obtained.

2.2 Quantitative real time PCR

DNA was extracted from 200 µl samples of whole blood or plasma with a DNA mini extraction kit (Qiagen, United Kingdom) according to the manufacturer's instructions. The extracted DNA was used immediately for amplification or was stored at -70°C until tested. Amplification and quantification were performed with the artus CMV PCR Kits (Qiagen, United Kingdom) with 4 quantitation standards. HCMV DNA was expressed as copies per milliliter. The principal uses of this HCMV molecular quantitative testing is for the diagnosis of active HCMV disease, which involves distinguishing patients with asymptomatic infection from patients with clinically significant HCMV disease.

2.3 Statistical analysis

Statistical comparisons of patient demographics were performed with chi-square tests for categorical variables. A p value <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Patient characteristics

A total of 200 transplant recipients consisting of 100 renal transplant recipients and 100 bone marrow transplant recipients were included in this study. The patient characteristics are listed in Table 1. The patients' ages ranged from 17 to 83 years for the renal transplant recipients and 78 patients were male. For the bone marrow transplant recipients, the ages ranged from 9 year to 83 years and 54 patients were male. The renal transplant recipients consist of 49% Malay, 23% Chinese, 14% Indians and 14% other indigenous races and the bone marrow transplant recipients consist of 74% Malay, 4% Chinese, 12% Indians and 10% other indigenous races.

Table 1: Characteristics of renal and bone marrow transplant recipients (January 2011 - June 2012).

	Renal transplant recipients		Mean Age	SD	Male/ Female
Characteristics	n=100	%	(Years)		(n/n)
Malay	49	49.0	51(26-83)	12.6	37/12
Chinese	23	23.0	40(17-72)	17.3	20/3
Indian	14	14.0	48(20-83)	20.4	10/4
Others	14	14.0	53(34-70)	9.4	11/3
	Bone marrow transplant recipients		**Mean Age**	**SD**	**Male/ Female**
Characteristics	n=100	%	(Years)		(n/n)
Malay	74	74.0	37(3-83)	23.5	39/35
Chinese	4	4.0	34(17-42)	11.3	2/2
Indian	12	12.0	30(9-73)	24.1	9/3
Others	10	10.0	48(11-83)	22.4	4/6

SD, Standard Deviation

3.2 Indications of renal and bone marrow transplantation

Transplant management has improved tremendously and most patients with kidney failure can be considered for transplantation. Bone marrow transplantation is used to treat a number of cancerous as well as non-cancerous conditions. The indications for renal and bone marrow transplantation in this study are listed in Table 2.

3.3 Incidence of HCMV infection by gender

The overall incidence of HCMV infection in the study group was 70.5% (141/200) where renal and bone marrow transplants account for 78.0% (78/100) and 63.0% (63/100) respectively. The incidence of HCMV infection in renal transplantation differed significantly by gender ($p<0.05$) where it was higher in males (78.2%) than in females (21.8%) but there was no statistically significant difference by sex in bone marrow transplantation in which males and females account for 60.5% and 39.5%, respectively.

Table 2: Indications of renal and bone marrow transplantation from January 2011 - June 2012.

Renal transplantation	n = 100	%	BM transplantation	n = 100	%
Glomerulonephritis	36	36	ALL	6	6
Diabetes Mellitus	13	13	Thalassemia major	6	6
Hypertension	24	24	Aplastic anaemia	5	5
Obstructive uropathy	2	2	AML	5	5
ADPKD	2	2	CML	5	5
Drugs/ toxic nephropathy	1	1	Hodgkin's lymphoma	0	0
Hereditary nephritis	0	0	Unknown	72	72
Unknown	22	22			

Abbreviations: BM, bone marrow, ADPKD, Autosomal Dominant Polycystic Kidney Disease; ALL, Acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia

3.4 Incidence of HCMV infection by races

There was no statistically significant difference ($p<0.05$) by races in both transplantation types as follows: 35% in Malay, 19% in Chinese, 13% in Indian and 12% in other indigenous races in renal transplantation, while 47% in Malay, 2% in Chinese, 5% in Indian and 8% in other indigenous races in bone marrow transplantation. The summary of HCMV incidence according to age, race and gender was tabulated in Table 3.

Table 3: Incidence of HCMV infections in renal and bone marrow transplant recipients in Malaysia from January 2011 – June 2012.

		n	(%)	Mean Age (Years)	Male/ Female (n/n)
Renal transplant recipients	Total	78/100	78	49(17-83)	
	Males	61/100	78		
	Females	17/100	22		
	Malay	35	45	51(34-83)	27/8
	Chinese	19	24	41(17-72)	16/3
	Indian	13	17	49(20-59)	9/4
	Others	12	15	54(46-70)	9/2
Bone marrow transplant recipients	Total	63/100	63	37(3-83)	
	Males	30/100	48		
	Females	33/100	52		
	Malay	47	75	39(3-83)	24/26
	Chinese	2	3	34(17-42)	2/0
	Indian	5	8	30(4-73)	5/0
	Others	8	13	48(11-83)	2/4

3.5 Incidence of HCMV disease

The incidence of HCMV disease was identified in 51% (40/78) of renal transplant recipients and 38% (24/63) of bone marrow transplant recipients. There is no statistical difference in the incidence of HCMV disease in renal and bone marrow transplant recipients. The most seen symptom in both groups was fever. The percentage of symptoms in both groups were presented in Table 4. All patients diagnosed with HCMV disease received treatment with intravenous ganciclovir, val-ganciclovir or acyclovir for a minimum of two weeks.

Table 4: Percentage of symptoms present in renal and bone marrow transplant recipients.

	Renal Transplant Recipients		BM Transplant Recipients	
	n=78	%	n=63	%
Fever	40	51.3	24	38.1
Lethargy	22	28.2	5	7.9
Headache	6	7.7	2	3.2

3.6 HCMV DNA load

The mean peak viral load in renal transplant recipients was 8,472,900 copies/ml (median 208,993; range 250-8,472,900 copies/ml). The mean peak viral load in bone marrow transplant recipients was 204,500 copies/ml (median 23,328; range 250-204,500 copies/ml). One renal transplant recipient was reported to have acute graft loss with repetitively high HCMV load in his plasma samples. The statistical significance of the HCMV viral load in renal and bone marrow transplant recipients in Malaysia from January 2011 – June 2012 are shown in Table 5.

Despite significant advances in the field of transplantation, infection remains the main cause of morbidity and mortality in both solid organ transplant and bone marrow transplant recipients. To date, HCMV is still one of the most serious pathogens in the field of transplantation [11].

Viral load of HCMV has been shown to be a major determinant factor in the severity and the manifestation of HCMV infection. Thus antiviral treatments were also provided for patient with high viral load of HCMV in this study. It correlates HCMV infection with decreased survival [12] and has become a measure of treatment success and surrogate for noncompliance and the presence of antiviral drugs resistance [13]. In this study population, HCMV disease was seen in 51% (40/78) renal transplant recipients and 38% (24/63) bone marrow transplant recipients. All of them were diagnosed of having more than 10,000 copies/ml up to 241,352,880,000 copies/ml of HCMV DNA. This shows that HCMV load is significantly higher in patients who develop disease thus signify the importance of viral load monitoring for patient interventions to prevent development of HCMV disease. In the context of response to therapy, the viral loads can decline immediately or may remain static for a period of time before reducing in copies. However in patients with a rapidly increasing HCMV load, they are more likely to exhibit a transient overshoot in HCMV load after therapy compared to those with a slower apparent growth rate [13]. Apart from this, if HCMV loads persist at high levels for greater than one week, the recommendations are that drug resistance against HCMV should be suspected [14].

Table 5: HCMV positivity of renal and bone marrow transplant recipients according to gender and ethnicity (January 2011 - June 2012).

		Viral Load (copies/mL)		p values
Renal transplant recipients		Mean	SD	
Gender				
	Males	253,993	1180660	0.9994
	Females	48,571	61904	
Ethnicity				
	Malay	342,649	1475222	0.036
	Chinese	199,127	573449	
	Indian	8,636	19813	
	Others	47,275	76839	

		Viral Load (copies/mL)		p values
Bone marrow transplant recipients		Mean	SD	
Gender				
	Males	32,462	26215	0.648
	Females	38,197	70080	
Ethnicity				
	Malay	23,078	52395	0.059
	Chinese	45,875	63816	
	Indian	2,350	3630	
	Others	35,375	82884	

SD, Standard Deviation

High frequency of HCMV infection is common in both solid organ transplant and bone marrow transplant recipients with the percentage of 60-70% [9, 15]. In our study patients, 78.0% renal transplant recipients were infected. In bone marrow transplant recipients, slightly lower percentage (63%) was infected by HCMV. There are three main races in Malaysia namely Malay, Chinese and Indian and many other indigenous races. Malay transplant recipients dominate the number of both type of transplantation followed by Chinese, other indigenous races and Indian. In renal transplant recipients, the incidence of HCMV infection are as follow; Malay (45%), Chinese (24%), Indian (17%) and others (15%). In bone marrow transplant recipients, the HCMV are as follow; Malay (75%), Chinese (3%), Indian (8%) and others (13%). The frequency of HCMV infection is closely related to several factors such as socio-economic background, donor and recipient's serostatus, patient management, late of HCMV onset and development of antiviral resistance in HCMV. In this study, we are not reporting the serostatus of the donors and recipients due to insufficient data. HCMV complications arise more frequently in patients known to be seropositive before transplantation usually as a result of reactivation of latent infection but occasionally due to *de novo* transmission from the transplanted organ or, transfused blood or blood products. Infection in seronegative recipients due to primary infection, while less frequent, is generally considered to be more severe [16]. The summary of patient classification based upon HCMV serostatus are: low risk (donor [D]-/recipient [R]-), intermediate risk (D+/R-), or high risk (D-/R+ or D+/R+) [17]. In the aspect of ethnicity, their serostatus before being transplanted were influenced by many factors, such as hygienic circumstances, socioeconomic factors, breastfeeding and sexual contact. Malaysian tends to have variations on these factors thus reflecting the HCMV serostatus among them throughout their lives. Gender is not considered a risk factor for developing CMV reactivation or disease [21]. Although high incidence of HCMV infection was seen in male patients in both renal and bone marrow transplant recipients, there is no explanation that gender has a direct effect in HCMV infection.

Host defences are the main factors that determine HCMV infection and the development of HCMV disease in an individual [1]. Several studies have demonstrated that major immune response against HCMV was carried out by cell-mediated immunity. Impaired of this arm of the immune response leads to severe infection. One study indicated that HCMV are able to avoid elimination by the immune system as the result of induction of a latent state of infection, exploitation of immunologically privileged tissue for replication and expression of genes that interfere with the immune response [18].

Symptomatic HCMV disease may occur at different times during immunocompromised state. It depends on the history of previous primary infection, the nature and the severity of the immunosuppression, exposure and the occurrence of graft-versus host disease (GVHD) [19]. In this study patients, 87% and 49% of renal and bone marrow transplant recipients respectively has developed HCMV disease. This could be, in part, attributed to the pre-emptive or prophylaxis therapy by the physician by acyclovir, ganciclovir and/or valganciclovir. Rubin and Cosimi [20], described the various syndromes caused by HCMV. Patel and Paya [21] also stated that the symptoms of HCMV infection in immunocompromised patients usually include prolonged fever, the presence of leukopenia and/or thrombocytopenia, and increased levels of serum transaminases. HCMV may also cause severe end-organ diseases, such as hepatitis, gastrointestinal infections and pneumonitis [21]. In this study, fever was found to be the most common manifestation of HCMV disease accompanied by systemic nonspecific complaints such as body malaise, anorexia, and myalgia [22]. Other common symptoms in this study were generalised lethargy and headache. Various complications including opportunistic infection either by bacteria, fungi or viruses were seen in immunocompromised patients. As an encounter measures several drugs and medication have been administered to the patients such as ceftriaxone, cefuroxime, cloxacillin, rocephin, tranexamic acid, ciprofloxacin, amphoteric drug, and others for the treatment of opportunistic infections.

Intravenous acyclovir, ganciclovir and valganciclovir have been used in this study population mostly for the pre-emptive strategy (where markers of HCMV replication trigger antiviral intervention) to control HCMV infection in

both renal and bone marrow transplant recipients. Prophylaxis therapy (where the antiviral drugs are given from transplantation for a defined duration –usually 100-200 days to cover the risk period for HCMV disease) were given to high risk patients [23]. The significant limitations of these drugs are drug toxicity and antiviral resistance. Pre-emptive therapy was preferred as it can minimise the unnecessary introduction of drugs to the patients plus it can lower the development of antiviral resistance [24].

For the past few years, quantitative polymerase chain reaction (PCR) assay has been the most commonly used methods in the diagnosis and management of HCMV infections in both solid organ and bone marrow transplant patients. We can predict the progress of HCMV infection by measuring HCMV DNA in serial blood leukocyte samples with this assay. Understanding the correlation between the viral load and the clinical symptoms has reduced the options of monitoring using qualitative methods. New quantitative molecular assays, including in house and commercial assays, have been described for the diagnosis of HCMV infection and monitoring of transplant patients [10, 25, 26].

4. CONCLUSION

More than 70% of HCMV detected in both renal and bone marrow transplant indicating that it is one of the major culprits in this condition and should not be neglected. The incidence of HCMV infection was slightly higher in renal transplant as compared to bone marrow transplant among Malaysian. This study also shows that HCMV viral load has no significant association with age, gender and HCMV disease. Various syndromes can be caused by HCMV ranging from a mild fever to severe end-organ diseases. Treatment with anti-HCMV therapy results in decline in HCMV load, usually to undetectable level.

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