ORIGINAL ARTICLE

Genotypic Distribution of Cytomegalovirus Infection and Associated Viral Load in a Tertiary Hospital in East Coast of Malaysia

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ABSTRACT

Introduction: Cytomegalovirus genotyping has been studied for its role in CMV pathogenesis and associated clinical manifestations. The aim of this study is to determine the genotypic distribution of CMV infection in a tertiary hospital and its associated viral load. **Materials and methods:** A total of 106 plasma samples from the patients suspected to have CMV infection based on their serological results between Jan 2021-Dec 2022 were included in the study and subjected for CMV DNA viral load. Only twelve positive samples out of 106 were then proceeded for genotyping study to detect eight CMV genotypes, (gB1-4; gH1-2, gL and gO). Patients' demographic and clinical data were retrieved from medical records. **Results:** Out of 106 plasma samples sent for CMV DNA viral load, twelve patients with positive results were subjected for detection of eight CMV genotypes. The commonest CMV genotype detected was gL, (n=7/12) followed by gB1, (n=5/12), gB4 (n=3/12), gO (n=2/12) and one for each other genotypes (gB2, gB3 and gH2) respectively. No patient had a positive gH1 gene. Compared to six patients who had only one CMV genotype found, four patients with combinations of CMV genotypes [CMV A (3 genotypes), D (2 genotypes), I (4 genotypes), and L (5 genotypes)] had higher viral loads. Two patients had no CMV genes detected. Patients with two or more genotypes detected have higher viral load. **Conclusion:** Patients with a combination of two or more genotypes (gB, gH/gL) had higher viral load possibly due to virus entry through activation of gB.
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Keywords: Genotypes, Cytomegalovirus, Viral load, Congenital CMV, Acquired CMV

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INTRODUCTION

Cytomegalovirus (CMV) is an enveloped virus under Herpesviridae family. It is a double-stranded DNA virus under β-herpesvirus subfamily. Among the human herpesviruses, it has the largest DNA genome which is around 235 kb pairs [1]. It is the commonest cause of congenital infection worldwide and causing severe disease in immunocompromised patients [2, 3]. Specific strains-related pathogenicity has been identified in few studies concerning both conditions. Therefore, genotyping plays an important role in discovering potential differences in pathogenicity of the disease and to discriminate between reinfection and reactivation in both immunocompromised host and pregnant lady.

CMV infection in pregnant women can either be primary or non-primary and the risk of fetal transmission is higher in primary compared to non-primary [4–6]. Besides, primary CMV infection in third trimester of pregnancy possesses higher risk of congenital transmission compared to non-primary [7]. According to Centers for Disease Control and Prevention (CDC), congenital CMV (cCMV) infection is defined as the detection of CMV DNA in the urine, saliva (preferred specimens), or blood, within three weeks after birth [8]. Diagnosis of cCMV can't be established if the samples taken after 3 weeks of life as it can't differentiate congenital or acquired CMV infection. However, saliva collected just after breastfed may cause false positive result, therefore, collection of saliva must be made at least 1 hour after breastfeeding [9]. Meanwhile in immunocompromised host, primary or reactivation of CMV infection can

cause severe disease for example in AIDS and transplant patients [10].

CMV has broad cell tropisms as it can replicate in many type of cells such as epithelial cells, endothelial cells, fibroblast and smooth muscle cells which facilitate systemic spread within the host and inter-host transmission [11]. There are several genes that encodes the CMV glycoprotein envelopes such as CMV UL75 that encodes glycoprotein H (gH), CMV UL115 (gL), CMV UL74 (gO) and CMV UL128 that encodes three structural proteins (pUL128, pUL130 and pUL131A) [12].

CMV used envelope glycoproteins to facilitate entry into host cell mediated by glycoprotein B (gB) and gH/gL [13]. gB as a Class III fusion protein of herpesviruses is responsible to help viral entry by mediating fusion of the virus and host membrane [14, 15]. Glycoprotein B (gB) of CMV that is encoded by UL55 gene accounts for more than 50% of CMV envelope. It has four major genotypes (gB1-gB4) .[16].It is an important determinant of viral virulence and plays an important roles in viral fusion and entry as well as virus transmission among cells. However, roles of gH/gL is unclear but a study proposed that gH/gL involved in viral entry process via gB activation [17].A study showed that gB/gH/gL formed stable complexes in extracellular virion independent of receptor binding [18].

gH/gL will be either part of trimeric complex with gO or pentameric complex with UL128/UL130/UL131A proteins that are abundant on HCMV envelope than gH/gL alone [19, 20]. These glycoprotein complexes are required for entry of CMV into fibroblasts, endothelial or epithelial cells and becoming targets of humoral immune response [19]. gO and UL128/UL130/UL131A proteins will bind through a disulfide bond with Gl-Cys144on gH/gL. The presence of mutations in UL128/UL130/UL131A of wild-type (WT) HCMV able to abolish epithelial or endothelial cell tropism that may happen spontaneously after only a few passages in fibroblasts [21, 22]. gH genotype is known to be associated with hearing loss and neurological dysfunction. gH1 genotype was known to be associated with hearing loss and neurological dysfunction while gH2 genotype reduce the risk of hearing loss [12, 23]. To date, there were four gL genotypes (gL1, gL2, gL3 and gL4) that has been identified [12].

The pentamer (gO/ UL128/UL130/UL131A) has been shown to be the main target of neutralizing humoral response to HCMV infection in epithelial and endothelial cells [24]. Strong neutralizing antibody response has been observed in animal studies such as in mouse, rabbit and rhesus macaque models following immunization with the pentamer and therefore served as main antigenic target for development of HCMV vaccine [25, 26]. To date, at least eight genotypes of gO identified (gO1-gO8) with 10 to 30% amino acids difference with two of them have five sub-genotypes. Previous studies showed that there were no correlation between gO genotypes and CMV disease and outcome of CMV infection in infants [12, 27].

There are many methods used for CMV genotyping such as nucleotide sequence analysis [28], restriction fragment length polymorphism (RFLP) [29, 30], real-time PCR [30, 31], deep-sequencing-based [32] and nested PCR. Nested PCR method was used in this study. Genotyping of CMV has shown several roles in previous studies. Few studies have described the correlations between CMV genotypes and disease severity [33, 34]. Apart from that, the most important role of CMV genotyping is to discriminate reinfection from reactivation in transplant patients and also in pregnant women to give an idea on donor-to-recipient transmission and mother-to-child transmission [31].

Focus of CMV genotyping is the envelope glycoproteins gB (UL55), gO (UL74) and gH (UL75) which are important for viral entry and major targets for neutralizing antibody responses [35]. Thus, the aim of this study is to determine the genotypic distribution of CMV infection in our centre and to relate them with the CMV DNA viral load.

MATERIALS AND METHODS

Study population

The study was performed on 106 plasma samples sent for CMV DNA viral load collected from patients who were suspected to have cytomegalovirus infection based on their CMV serology results that were positive either for CMV IgM or IgG between January 2021 and December 2022. The samples were submitted to the molecular laboratory Department of Medical Microbiology and Parasitology at the Universiti Sains Malaysia. Samples that were tested positive for CMV viral load were proceeded for genotyping study. Patients' age and clinical diagnosis were retrieved from medical records. The following data were collected from the patients' medical records: age at the time blood taken for CMV diagnosis, clinical presentation and diagnosis. Apart from that, for patients less than one year of age, data on audiological assessment, ophthalmology and paediatric follow up were also obtained.

Operational Definition

Congenital CMV infection

According to CDC, congenital CMV infection (cCMV) is defined as detection of CMV DNA in the urine, saliva (preferred specimens), or blood, within three weeks after birth.[8] However, since seropositive mothers can shed CMV in breastmilk and cause false positive result with saliva sample, urine is the preferred specimen despite the fact that it is advised to wait around an hour after

breastfeeding before collecting saliva.[9]

Acquired CMV infection
After exclusion of congenital CMV infection, it is regarded as acquired CMV infection.

Recent infection
Samples taken two weeks apart that show evidence of seroconversion can be used to diagnose recent CMV infection.[36]

DNA extraction
Nucleic acid extraction was done previously by using GeneProof Pathogen Free DNA Isolation Kit (GeneProof, Czech Republic), following the manufacturer's guidelines. Initially, patient whole-blood collected in BD Vacutainer EDTA tube (Becton Dickinson, USA) was centrifuged at 1,500 ×g for 10 minutes to obtain the plasma fraction. Two hundred microliter of plasma from each patient was subjected to nucleic acid extraction, giving the elution volume of 100 µl per sample.

Real-time polymerase chain reaction (qPCR) for CMV
CMV PCR kit (GeneProof, Czech Republic) was used for detection of human CMV nucleic acid. The 40 µl total volume of a single reaction tube consisted of 30 µl master-mix buffer and 10 µl of the extracted nucleic acid sample. Amplification condition was set up at 37°C for 2 minutes, 95°C for 10 minutes, followed by a 45-cycle of 95°C for 5 seconds, annealing at 60°C for 40 seconds, and 72°C for 20 seconds. CMV positive was indicated by amplification curve at the FAM detection channel, corresponding to the presence of exon 4 immediate-early antigen CMV gene, together with an internal control amplification curve at the HEX channel. CMV viral loads were determined in the international units per milliliter (IU/ml).

Identification of CMV genotypes by PCR
CMV genotypes for gB (UL55), gH (UL75), gO (UL74) and gL (UL115) genes were identified from the patients' DNA samples with positive qPCR for CMV viral loads. Genotyping of CMV for gB (UL55) genotypes 1 to 5 was done by using a multiplex nested PCR as described by Tarrago et al., 2003 [37]. Briefly, the highly polymorphic region of CMV gB gene was initially amplified using the external primers, CMVQ1F and CMVQ1R in the first round PCR. The 25 µl of PCR mixture contained 5 µl of 2× MyTaq Red DNA polymerase buffer (Bioline), 1 pmol/µl of each primer, 0.2 µl of *Taq* DNA polymerase 1 unit/µl, 5 µl of DNA template and ultra-pure water. PCR amplification consisted of initial denaturation at 95°C for 3 minutes; a 45-repeating cycle of 95°C for 30 seconds, annealing at 60°C for 30 seconds, and 72°C for 30 seconds; and final elongation at 72°C for 5 minutes.

One microliter of the first round amplified product was used in the second round PCR using CMVGT 1F to CMVGT 5F sense and CMVQ2 R antisense primers. Other PCR components and amplification condition were set similar to the first round, except that the annealing temperature used was 58°C for 1 minute. CMV genotyping for gO (UL74) and gL (UL115) were also done by nested PCR as described by Paradowska E. et al., 2019.[12] The PCR components and conditions were similar to that of gB genotyping. Genotype variations for gO and gL were identified by the presence of amplicons at 356 bp and 558 bp, respectively. Meanwhile, the presence of CMV gH genotypes (UL75) was identified by singleplex PCR for gH1 and gH2, by the presence of amplicons at 181 bp and 134 bp, respectively, as described by Nahar S. et al, 2018 [38]. All the primers and annealing temperature used for PCR CMV genotyping are listed in Table I. PCR product were electrophoresed on 2% agarose gel as seen in Figure 1.

Table I: Details of primer sequences and PCR amplifications of CMV gB, gH, gL and gO genotyping.

Target gene/ amplicon size	Primer sequence (5′ to 3′)	Annealing temperature	References
CMV gB (UL55) 751 bp	CMVQ1 F: TTTGGAGAAAACGCCGAC CMVQ1 R: CGCGCGGCAATCGGTTTGTTGTA	60°C	
gB1 420 bp	CMVGT 1F: ATGACCGCCACTTTCTTATC		
gB2 613 bp	CMVGT 2F: TTCCGACTTTGGAAGACCCAACG		Tarrago et al., 2003[32]
gB3 190 bp	CMVGT 3F: TAGCTCCGGTGTGAACTCC		
gB4 465 bp	CMVGT 4F: ACCATTCGTTCCGAAGCCGAG-GAGTCA	58°C	
gB5 139 bp	CMVGT 5F: TACCCTATCGCTGGAGAAC		
gB1-5	CMVQ2 R: GTTGATCCACRCACCAGGC		
gH1 (UL75) 181 bp	gH1 F GAGACTTAACACCTACGCAT gH1 R CGATCCCTTCCAGTCG	55°C	Pa-ca-Uc-cara-lertkun et al., 2013[34]
gH2 (UL75) 134 bp	gH2 F TGGACACGATCTACTATTCA gH2 R TGTCGTCGTCTATGGAC	55°C	Nahar S. et al, 2018[33]
gO (UL74) 868 bp	UL74-gO F TAACGGGCGCTTGTTTACGT UL74-gO R CAGCAAAACGACCAGAATCAG	68°C	Paradowska E. et al., 2019[9]
gO (UL74) 356 bp	UL74-gO-nested F TAGATTCCGGCTCATGGCGTT UL74-gO-nested R CCCAGCTTAGAAAACCCGCAA	66°C	
gL (UL115) 636 bp	UL115-gL-F GACGCACGGCGCGGTTGGTACG UL115-gL-R CGTGCCGCAGACTTGATGTGC-CG	55°C	Paradowska E. et al., 2019[9]
gL (UL115) 558 bp	UL115-gL-nested F CGGTGGCACCAGCTCGAAGCCT UL115-gL-nested R ATGTGCCGCCGCCCGGATT	55°C	

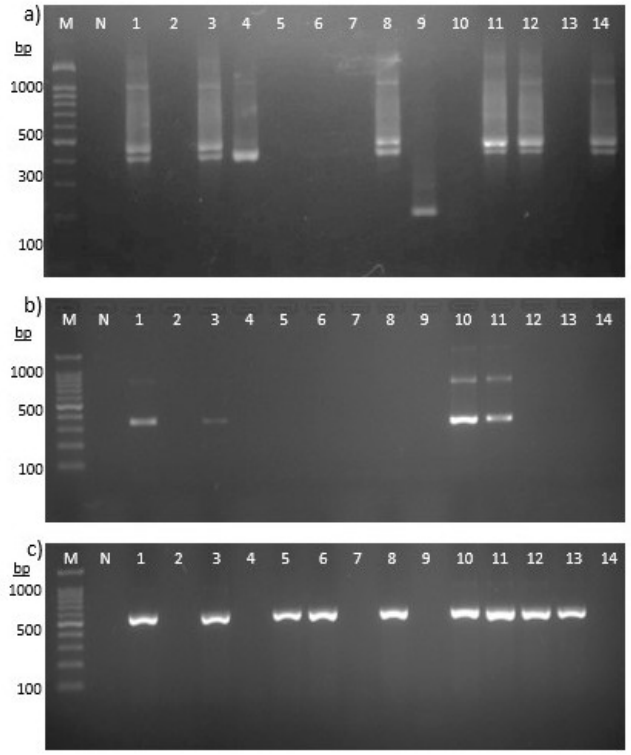

Figure 1: Agarose gel image of PCR amplifications for a) gB, b) gO, and c) gL genotypes. Lane M: 100 bp DNA ladder, lane N: negative control, lane 1-14: PCR amplicons from patients with CMV infection.

Statistical analysis

The data were analyzed using descriptive statistic due to very small number of samples obtained for 2 years.

Ethical approval

This study has received an ethical approval from human ethic with the JEPeM Code of USM/JEPeM/17110606).

RESULTS

From a total of 106 samples, 12 were positive for CMV DNA viral load (11.3%). These patients were enrolled in this study for further CMV genotypic characterization. Of these, 83.3% (n = 10/12), gB, gH, gL and gO genotypes were successfully identified by PCR analysis (Figure I).

The commonest CMV genotype detected was gL, (n=7/12) followed by gB1, (n=5/12), gB4 (n=3/12), gO (n=2/12) and one for each other genotypes (gB2, gB3 and gH2) respectively. No patient had a positive gH1 gene. Four patients have a combinations of CMV genotypes [CMV A (3 genotypes), D (2 genotypes), I (4 genotypes) and L (5 genotypes)] while remaining six patients have only one CMV genotype detected. However, CMV genotype can't be detected in two patients. CMV L patient with the combination of four genotypes has the highest CMV viral load followed by CMV D, CMV I and CMV A patients (Refer to Table III).

Age and clinical diagnosis of the patients were presented in Table II. The ages ranged between Day 16 of life and 54 years old, with more than half (66.7%) of the study participants are among children less than one year of age. Based on the clinical data, only one patient, CMV C fulfilled the definitive criteria of congenital CMV (cCMV) infection as the blood sample for CMV DNA PCR sent within 3 weeks of life which refers to operational definition by CDC above and 2 patients confirmed to have acquired CMV infection.

Table II: Age and clinical diagnosis of study participants (n=12)

No.	Patient's ID	Age of CMV DNA detection	Diagnosis
1	CMV A	2 months old	Direct hyperbilirubinemia with transaminitis secondary to CMV infection
2	CMV B	1 month and 8 days old	Asymmetrical small for gestational age (SGA) with sepsis and underlying CMV infection
3	CMV C	Day 16 of life	Congenital CMV infection with enterocolitis
4	CMV D	2 months and 5 days old	Thrombocytopenia secondary to Acquired CMV infection
5	CMV E	1 month and 26 days	Small for gestational age (SGA) with sepsis and underlying CMV infection
6	CMV F	3 months old	Prolonged jaundice with neonatal hepatitis secondary to CMV infection
7	CMV G	8 months old	Langerhan cell histiocytosis
8	CMV H	5 months old	Acquired CMV infection
9	CMV I	4 years old	Severe autosomal recessive Purine Nucleoside Phosphorylase (SCID) post hematopoietic stem cell transplant (HSCT) with CMV reactivation. Given IV Ganciclovir for 2 weeks.
10	CMV J	6 years old	B-Acute lymphoblastic leukemia (B-ALL) with CNS involvement
11	CMV K	33 years old	Acute myeloid leukemia (AML) post allogeneic stem cell transplant complicated with acute hepatitis secondary to CMV reactivation. Given IV Ganciclovir for 2 weeks.
12	CMV L	54 years old	Recurrent CMV ColitisGiven oral Valganciclovir for 6 weeks.

However, CMV A, B, E and F patients were also put under multi-disciplinary team management to monitor CMV complications follow up such as ophthalmology to rule out CMV retinitis, audiology to rule out sensorineural hearing loss and paediatric for developmental milestones assessment even though blood for CMV DNA PCR were taken after 3 weeks of life as cCMV can't be ruled out in these cases. The possibility of the patients having cCMV were there and they might get long-term complications of cCMV such as sensorineural hearing loss, developmental delay and CMV retinitis. CMV DNA taken within 3 weeks of life as CMV screening is

not a routine practice in asymptomatic neonates. Thus, the possibility of having cCMV in these patients might be there and may indicate possibility of high burden of CMV infection transmitted vertically.

By referring to Table III, CMV serology results that were traced from these patients showed that two patients have evidence of seroconversion that indicate recent CMV infection (CMV D & H) from different samples sent more than 2 weeks apart. However, three patients did not sent second samples to demonstrate evidence of seroconversion.

Table III: CMV genotypes detected in the study participants (n=12)

No.	Patient's ID	CMV Serology	CMV Serology Result Interpretations	Viral load (IU/ml)	Genotypes detected								Outcome
					gB1	gB2	gB3	gB4	gH1	gH2	gL	gO	
1	CMV A	(1st sample) CMV IgM: Reactive CMV IgG: Reactive (181.8 U/ml) No second sample sent	Unable to interprete	3293	√	√	ND	ND	ND	ND	√	ND	Alive
2	CMV B	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (189.3 U/ml) (2nd sample- 2 weeks apart) CMV IgM: Non-reactive CMV IgG: Reactive (208.9 U/ml)	No evidence of seroconversion	452	ND	ND	ND	ND	ND	ND	√	ND	Alive
3	CMV C	(1st sample) CMV IgM: Reactive CMV IgG: Reactive (67.34 U/mL) (2nd sample- 2 weeks apart) CMV IgM: Non- reactive CMV IgG: Reactive (42.32 U/mL)	No evidence of seroconversion	1532 (Congenital CMV infection)	ND	ND	ND	ND	ND	ND	√	ND	Alive
4	CMV D	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (286.31 U/ml) (2nd sample- 2 weeks apart) CMV IgM: Reactive CMV IgG: Reactive (96.06 U/ml)	Evidence of seroconversion Recent CMV infection	36482 (Acquired CMV infection)	√	ND	ND	√	ND	ND	ND	ND	Alive
5	CMV E	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (201.8 U/ml) (2nd sample- 2 weeks apart) CMV IgM: Non- reactive CMV IgG: Reactive (226.3 U/ml)	No evidence of seroconversion	813	ND	ND	ND	ND	ND	ND	ND	ND	Alive
6	CMV F	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (118.8 U/ml) (2nd sample- 2 weeks apart) CMV IgM: Non-reactive CMV IgG: Reactive (77.60 U/ml)	No evidence of seroconversion	1936	ND	ND	ND	ND	ND	ND	√	ND	Alive
7	CMV G	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (13.92 U/ml) No second sample sent	Unable to interprete	221	ND	ND	ND	ND	ND	ND	√	ND	Alive
8	CMV H	(1st sample) CMV IgM: Indeterminate CMV IgG: Reactive (19.12 U/ml) CMV IgM: Reactive CMV IgG: Reactive (18.04 U/ml)	Evidence of seroconversion Recent CMV infection	678 (Acquired CMV infection)	ND	ND	ND	ND	ND	ND	ND	ND	Alive
9	CMV I	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (96.22) U/ml (2nd sample- 2 weeks apart) CMV IgM: Non-reactive CMV IgG: Reactive (79.86) U/ml	No evidence of seroconversion	22455	√	ND	ND	√	ND	ND	√	√	Alive
10	CMV J	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (147.1) U/ml (2nd sample- 2 weeks apart) CMV IgM: Non-reactive CMV IgG: Reactive (166.5) U/ml	No evidence of seroconversion	<122	√	ND	ND	ND	ND	ND	ND	ND	

CONTINUE

Table III: CMV genotypes detected in the study participants (n=12). (CONT.)

No.	Patient's ID	CMV Serology	CMV Serology Result Interpretations	Viral load (IU/ml)	Genotypes detected								Outcome
					gB1	gB2	gB3	gB4	gH1	gH2	gL	gO	
11	CMV K	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (955.9) U/ml No second sample sent	Unable to interprete	4622	ND	ND	√	ND	ND	ND	ND	ND	Passed away due to septic shock secondary to pneumonia with AML in crisis
12	CMV L	(1st sample) CMV IgM: Non-reactive CMV IgG: Reactive (85.96) U/ml (2nd sample- 2 weeks apart) CMV IgM: Non-reactive CMV IgG: Reactive (193.9) U/ml	No evidence of seroconversion	965,600	√	ND	ND	√	ND	√	√	√	Alive

ND: Not detected

DISCUSSION

CMV infects nearly 40-100 % adults worldwide and causing congenital infection as well as morbidity and mortality among immunosuppressed individuals. It is estimated that around 20-30% of nonimmune pregnant women infected with CMV transmit the virus to their offspring, whilst rate of transmission is lower, 0.6-1% following nonprimary CMV infection.[39] Thus, congenital CMV (cCMV) is the leading infectious causes of developmental delay and sensori-neural hearing loss (SNHL) worldwide.[40]

CMV infections in humans generally show mild symptoms or are asymptomatic making the early diagnosis troublesome. However, patients with cCMV may present with several clinical signs either at birth such as rash, jaundice, microcephaly, low birth weight, hepatosplenomegaly, seizures, retinitis or long-term health problems such as sensorineural hearing loss or developmental delay.[41]

In this study, out of eight patients with less than one year of age, only one patient (CMV C) was diagnosed as cCMV and was put under multi-disciplinary team management . The blood for CMV DNA for this patient was taken at day 16 of life which was less than 3 weeks after birth and fulfilled criteria for cCMV diagnosis by CDC for this patient.[8] Serological diagnosis is not meaningful for patients with less than one year of age as the patient may acquire antibodies (such as IgG) transplacentally. A study showed that gL3 and gO4 genotypes are important congenital infection virological marker,[12] however, in this patient (CMV C), only gL genotype was positive but negative for gO genotype.

Even though blood for CMV DNA were taken after 3 weeks of life for these patients (CMV A, B, E and F), they were put under multi-disciplinary team management too as CMV screening is not a routine practice here and also worldwide.[9] Possibility of cCMV might be there in these patients.

By referring to Table III, two patients, CMV D and CMV H were diagnosed with recent acquired CMV infection by evidence of seroconversion from negative or intermediate CMV IgM to positive CMV IgM following CDC guideline.[36]

Consistent with Manuel et al., patients with mixed gB genotypes exhibited higher viral loads, supporting the association between mixed genotypes and increased viral burden. [42] Out of 6 patients that positive for gB genotypes, 4 of them had mixed genotypes (CMV A: gB1 and gB2 ; CMV D, I and L : gB1 and gB4) and two with only gB1 genotype (CMV J) and gB3 genotype (CMV K). Those with mixed gB genotypes showed higher viral loads and in agreement with previous aforementioned study.

While gB responsible for virus-host membrane fusion during viral entry, the role of gH/gL in fusion process is unclear. Only one patient had gL genotype together with gB1 and gB2. Four patients (CMV B, C, F and G) had mixed genotypes (gH1, gH2 and gL). From our result, patients with a combination of two or more genotypes had higher viral load (CMV A : gB1, gB2, gL ; CMV D : gB1, gB4, gH1, gH2; CMV I: gB1, gB4, gL and gO; CMV L: gB1, gB4, gH2, gL and gO) as it has been proposed in previous study that gH/gL has been involved in the virus entry process via activation of gB.[17]

Table II showed that no CMV genotypes detected for two patients (CMV E and H). This is probably due to the presence of other genotypes that were not tested in this study such as glycoprotein N (gN-1, gN-2, gN-3a, gN-3b, gN-4a, gN-4b, gN-4c, and gN-4d) that was encoded by CMV UL73 that may be present in these patients, [28, 43] or (pUL128/pUL130/pUL131A complexes) that was encoded by CMV UL128 locus.[12]

Few studies showed that infection with more than one CMV genotypes in solid organ transplant recipients has negative outcomes, however to date, there is no recent findings for recipients of hematopoietic stem

cells transplant [16, 42]. Two patients developed CMV reactivation post hematopoietic stem-cell transplant and started on IV Ganciclovir. One patient with a combination of CMV genotypes (gB1, gB4, gL, gO) survived and another one with single genotype (gB3) passed away 2 months after completed IV Ganciclovir due to AML in crisis with pneumonia.

This study has several limitations. In Malaysia, there hasn't been any research done on distribution of CMV genotype yet. Therefore, it was quite difficult to compare the distribution of genotypes with other centres locally. Apart from that, we just managed to get only 12 positive cases of CMV viral load in 2 years. In order to get additional samples and obtain a more comprehensive picture of the circulating genotypes in Malaysia, the study's duration needs to be extended. We are unable to further differentiate between these genotypes, gL; (gL1-4); gO (gO1-8) and others that may be missed in all patients, including two patients that documented no tested genotypes due to financial constraints.

CONCLUSION

In conclusion, patients with a combination of two or more genotypes (gB, gH/gL) had higher viral load possibly due to virus entry through activation of gB in comparison to patients with only one genotype detected.

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