ORIGINAL ARTICLE

Distribution of Cytomegalovirus Glycoprotein B Genotypes Among Renal and Haematopoietic Stem Cell Transplant Recipients in a Tertiary Hospital, Malaysia

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ABSTRACT

Introduction: Cytomegalovirus (CMV) glycoprotein B (gB), encoded by gpUL55, is crucial for CMV's cellular entry and a potential pathogenicity marker. We investigated CMV gB genotype distribution in renal and haematopoietic stem cell transplant (HSCT) recipients, assessing correlations with disease. **Materials and methods:** 264 clinical samples from 110 renal and 154 HSCT recipients at a tertiary hospital in Kuala Lumpur, Malaysia were analysed. Quantitative PCR detected four CMV gB genotypes (gB1-4), and clinical data correlations were assessed. CMV serostatus of donors (D) and recipients (R) was determined pre-transplantation. **Results:** In renal transplant recipients, 48.2% exhibited single-genotype CMV, with gB2 (20.9%) and gB1 (17.2%) most prevalent. HSCT recipients showed 46.1% single-genotype CMV, primarily gB2 (23.4%) and gB1 (16.2%). Mixed-genotype infections were observed in 51.8% of renal transplant and 53.9% of HSCT recipients, particularly gB1-gB2 (65.8%). Mixed gB genotypes showed significant CMV disease association in HSCT recipients ($p < 0.05$) but not in renal transplant recipients. Virus load comparisons indicated no significant differences in both groups, but renal transplant recipients with mixed infections had a higher median viral load. The majority of both recipient groups were D+/R+ (84.5% renal, 79.2% HSCT). Primary diagnoses among recipients varied, including glomerulonephritis, diabetes mellitus, hypertension, acute leukemias, lymphomas, and other conditions. **Conclusion:** This study reveals the diversity of CMV genotypes in renal and HSCT transplant recipients and their potential impact on disease correlation, providing insights into genotype prevalence, viral load association, and CMV disease risk.
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Keywords: Cytomegalovirus (CMV), Genotypes, Glycoprotein B, Renal transplant, Haematopoietic stem cell transplant (HSCT), CMV disease

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INTRODUCTION

Cytomegalovirus (CMV) is a common opportunistic pathogen that can cause significant morbidity and mortality in immunocompromised individuals, such as those undergoing renal and haematopoietic stem cell transplantation (HSCT) (1). CMV infects a wide range of cell types leading to end-organ disease, including pneumonitis, hepatitis, retinitis, and gastrointestinal disease (1). To enter different cell types, different forms of glycoprotein are utilized such as gB, gH/gL, and gO (2). CMV glycoprotein B (gB) (UL55) is a major envelope glycoprotein, the fusion protein that participates in virus entry (2). It exists in the viral envelope and acts as a protein dimer causing lysis of protein on the membrane surface of all CMV-infected cells. gB is also essential for cell-to-cell proliferation by priming the transcriptional machinery in the host cell before viral replication commences. gB gene is a potential marker for CMV virulence, tissue tropism, or other pathogenicity (3).

Renal and HSCT recipients are particularly susceptible to CMV infection despite HLA matching between donors and recipients, due to the immunosuppressive regimens they receive to prevent graft rejection (4,5).

The distribution of CMV gB genotypes among renal and haematopoietic stem cell transplant recipients in a tertiary hospital in Malaysia has not been extensively studied. The finding of CMV gB genotypes distribution in the population may provide insights into the pathogenicity of different gB genotypes relates with clinical manifestations and outcomes (6). As certain gB genotypes may be more immunogenic, potential gB may potentiate as target for vaccine development. The information regarding its pathogenicity may also induce the diagnostic and therapeutic strategies for CMV infection in transplant recipients (7). Despite the extensive research on CMV infection and its association with renal and haematopoietic stem cell transplantation, there is still a gap in knowledge regarding the distribution of CMV gB genotypes among transplant recipients in Malaysia. Most of the existing studies have focused on other populations and geographical regions (4,5,8,9,10,11,12,13). In this study we determined the distribution of the CMV gB genotypes among renal and haematopoietic stem cell transplant recipients in a tertiary hospital in Malaysia investigated the differences in the clinical characteristics of the patients according to their different CMV genotypes.

MATERIALS AND METHODS

Study population and sample collection

In this research, we employed a retrospective study design. Due to the limited number of renal transplant and HSCT patients, we used a convenience sampling method for sample collection. The study included a total of 264 patients, consisting of 110 renal transplant recipients and 154 allogeneic haematopoietic stem cell transplant (HSCT) recipients from a tertiary hospital in Kuala Lumpur between January 2017 and December 2022. Renal transplant recipients underwent monthly CMV viral load monitoring for six months, with additional assessments at months nine, twelve, eighteen, and twenty-four. HSCT patients had weekly CMV viral load monitoring from infusion to day 100 post-HSCT, followed by monthly checks for up to one year.

The inclusion criteria for the study were as follows: (1) individuals who had received a renal or haematopoietic stem cell transplant, and (2) individuals who have tested positive for CMV from their plasma samples. In the genotyping analysis, we selected samples based on their viral load. This selection method was preferred because higher viral loads enhance the reliability of obtaining precise genotyping results. All archived plasma samples of 264 patients were collected and stored at -20 °C. Medical records and laboratory request form of each participant were then reviewed to obtain clinical and demographic data, including age, sex, ethnicity, underlying medical conditions, and medication history.

Laboratory test for HCMV infection

CMV DNA isolation and detection

DNA extraction from 264 plasma was performed using the QIAamp DNA Mini Kit (Qiagen, Germany) following the blood and body fluid protocol as instructed in the supplied user manual. Prior to polymerase chain reaction (PCR), the purity and concentration of the extracted DNA were assessed using a spectrophotometer (BioRad SmartSpec Plus Spectrophotometer) to ensure high-quality DNA samples were obtained for analysis. CMV viral load testing was conducted using the Artus® CMV RG PCR test kit (Qiagen, Germany) following the manufacturer's instructions. A standard curve was established utilising the four CMV positive controls provided in the kit, enabling accurate quantitation of CMV viral load in the samples. To ensure reliable results, a negative CMV control was included to exclude the possibility of false-positive CMV. In the testing process, 20 µL of DNA sample eluate was combined with 30 µL of the working master mix. The resulting amplicons were detected by measuring fluorescence with the Rotor-Gene Q Thermocycler (Qiagen, Germany) under the following amplification conditions: an initial step at 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 55°C for 1 minute. Upon completion of amplification, the data were analyzed using the Rotor-Gene Q software package. The linear range of the CMV DNA test spanned from 2.3 to 5.7 log10 copies/mL. Quantitative results were reported for measurements exceeding 2.3 log10 copies/mL (200 copies/mL), while positive results below this threshold were reported as <200 copies/mL, of CMV DNA detected.

CMV genotyping

Genotypes gB1, gB2, gB3 and gB4 were identified by real-time PCR using Qiagen SYBR green qPCR Master mix (Qiagen, Germany) as per manufacturer guidelines. Post-thaw, solutions were vortexed and centrifuged. The 25µl PCR mixture consisted of 12.5µl Master Mix, 0.75µl primers, 6.75µl nuclease-free water, and 5µl template DNA. Two housekeeping genes, Ribosomal Protein L22 (RPL22) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were utilised as internal controls to verify the accuracy of the PCR process. A non-template control was included as the negative control to establish a reference point for the absence of the expected response. The control mixture (25µl) contained 12.5µl Master Mix, 0.2µl primers, 7.1µl RNase-free water, and 5µl template DNA. Reactions were mixed, aliquoted, and centrifuged in PCR tubes. Real-time PCR was conducted using a C1000 Touch Thermal Cycler (Bio-Rad CFX96) under these conditions: initial denaturation at 95°C for 10 min; 40 cycles of denaturation at 95°C for 15 s, annealing at 42.5°C for gH and 44.5°C for gN for 30 s, and extension

at 72°C for 30 s. Post-cycling, a melting curve analysis was performed. The following primers were used: gB1 forward (5′-CATACGACGTCTGCTGCTCACT-3′) and gB1 reverse (5′-GCTGACCGTTTGGGAAGAAG-3′), gB2 forward (5′-TCTTTGGTGGAATTGGAACGT-3′) and gB2 reverse (5′- TGTCACTCG TACTTCTTCTGGTCCTA-3′), gB3 forward (5′-TGTTGGAACTGGAACGTTTGG-3′) and gB3 reverse (5′-TGCCCGTACTTCTCTTGGTTCT-3′), and gB4 forward (5′- AAACGTGTCCGTCTTCGAAACT-3′) and gB4 reverse (5′- TCCACCAGAGATTTTTGCTTGA-3′) (Manuel et al., 2009).

Statistical analyses

Statistical analysis was conducted using the SPSS ver. 25.0 software (SPSS, Inc., Chicago, IL, USA). The distribution of gB genotypes among renal and haematopoietic stem cell transplant recipients, the relationship between the gB1, gB2, gB3, and gB4 genotypes, viral load, CMV disease, and acute graft rejection was analysed using chi-square tests, ANOVA, Mann-Whitney U-Test, and the Kruskal-Wallis test. A p-value <0.05 was considered to be statistically significant. CMV disease is a manifestation of various clinical presentations such as colitis, CMV hepatitis, esophagitis, CMV gastrointestinal disease, diarrhoea, acute gastritis, CMV viral syndrome, and CMV pneumonitis.

Ethical Clearance

This study was reviewed and approved by the Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia (approval number: NMRR-20-993-53201).

RESULTS

Patient demographics

The demographic characteristics of 264 participants are summarised in Table I. The mean age of the renal transplant recipients was 42.7 years, ranging from 13 to 68 years. The mean age of the HSCT recipients was 26.1 years, ranging from 9 to 31 years. The primary diagnosis for renal transplant recipients were glomerulonephritis (36%), diabetes mellitus (13%), hypertension (24%), obstructive uropathy (2%) autosomal dominant polycystic kidney disease (ADPKD) (2%), drugs/toxic nephropathy (1%), and unknown (22%). The primary diagnosis for HSCT recipients were acute leukaemias (27%), chronic myelogenous leukaemia (CML) (2%), lymphomas (30%), myelodysplastic syndrome (MDS) (2%), myeloproliferative syndrome (MPS) (1%), multiple myeloma (5%) and unknown (33%). The primary diagnosis of both transplant recipients were presented in Table II. Immunosuppressive regimens were administered to transplant recipients, and the management of graft rejection cases followed established protocols.

Table I: Demographic of 264 immunocompromised patients with cytomegalovirus (CMV) infection.

Characteristic	Renal transplant recipients (n=110)	HSCT recipients (n=154)
Mean age (range), years	42.7 (13 - 67)	26.1 (9 - 31)
Male/Female, (%/%)	58/48	56/44
CMV Serostatus, n (%)		
D+/R+	93 (84.5)	122 (79.2)
D+/R-	10 (9.1)	18 (11.7)
D-/R+	5 (4.5)	8 (5.2)
D-/R-	2 (1.8)	6 (3.9)

Table II: Primary diagnosis of renal and haematopoietic stem cell transplant recipients from January 2017 - June 2022

Renal transplant recipients	n = 110	%	HSCT transplant recipients	n = 154	%
Glomerulone-phritis	40	36	Acute leukaemias	42	27
Diabetes mellitus	14	13	CML	3	2
Hypertension	26	24	Lymphomas	46	30
Obstructive urop-athy	2	2	MDS	3	2
ADPKD	2	2	MPS	2	1
Drugs/ toxic ne-phropathy	1	1	Multiple myeloma	8	5
Unknown	24	22	Unknown	51	33

Abbreviations: ADPKD, Autosomal Dominant Polycystic Kidney Disease; CML, Chronic myelogenous leukaemia;
MDS, myelodysplastic syndrome; MPS, myeloproliferative syndrome.

CMV infection rates

Cytomegalovirus (CMV) infection was a significant concern among transplant recipients. Primary CMV infection, defined as infection in a previously seronegative recipient (D+/R-), occurred in 9.1% (n=10) of renal transplant recipients and 11.7% (n=18) of HSCT recipients. CMV reactivation, occurring in seropositive recipients (D-/R+ or D+/R+), was more common, observed in 89% (n=98) of renal transplant cases and 84.4% (n=130) of HSCT cases.

Detection of gB genotypes in clinical samples

We observed a single-genotype infection in 53 (48.2%) and 71 (46.1%) among 110 renal transplant recipients and 154 HSCT recipients respectively. As in the Table III, among 110 renal transplant recipients, gB2 showed the highest genotypes detected [19/110 (20.9%)] followed by gB1 [23/110 (17.2%)], gB3 [8/110(7.2%)] and gB4 [3/110 (2.7%)]. Similarly in 154 HSCT samples, gB2 was also the highest genotype detected [36/154 (23.4%)] followed by gB1 [25/154 (16.2%)], gB3 [6/154 (3.9%)] and gB4 [4/154 (2.6%)].

Table III: Cytomegalovirus (CMV) glycoprotein B (gB) genotype and CMV load in 264 plasma samples obtained from immuno-compromised patients (renal transplant and HSCT recipients).

Type of transplantation CMV genotype(s) isolated	No of samples, n (% from renal transplant or HSCT)	Median (IU/mL)	Range (IU/mL)
Renal transplant recipients (110)			
Single genotype			
gB1	19 (17.2)	53,350	250–185,700
gB2	23 (20.9)	52,540	250–167,760
gB3	8 (7.2)	33,205	250– 62,500
gB4	3 (2.7)	18,750	16,478– 20,250
Mixed genotypes			
gB1-gB2	36 (32.7)	58,625	250– 224,550
gB1-gB3	7 (6.3)	62,560	18,680– 189,500
gB2 and gB3	12 (10.9)	54,754	635– 197,100
gB2 and gB4	2 (1.8)	147,720	129,640– 165,800
HSCT recipients (n=154)			
Single genotype			
gB1	25 (16.2)	494	250– 269,976
gB2	36 (23.4)	1,195	250– 666,297
gB3	6 (3.9)	693	250– 7,486
gB4	4 (2.6)	396	250– 497
Mixed genotypes			
gB1-gB2	51 (33.1)	5,998	250– 792,667
gB1-gB3	13 (8.4)	7,737	495 – 83,696
gB2 and gB3	15 (9.7)	5,095	635– 197,100
gB2 and gB4	4 (2.6)	1,330	250– 2,995

Mixed B genotypes were identified in 57/110 (51.8%) samples of renal transplant recipients and 83/154 (53.9%) HSCT recipients. In renal transplant recipients, gB1 and gB2 were the most common combination observed in 36 samples from 110 (32.7%), followed by gB1 and gB3 in 7 samples (6.3%), gB2 and gB3 in 12 samples (10.9%), and gB2 and gB4 in 2 samples (1.8%). In haematopoietic stem cell transplant (HSCT) recipients out of 154 samples, the most frequent dual gB genotypes identified were gB1 and gB2 in 51 samples (33.1%), gB1 and gB3 in 13 samples (8.4%), gB2 and gB3 in 15 samples (9.7%), and gB2 and gB4 in 4 samples (2.6%). The distribution of these genotypes was represented in Figure 1, illustrating the respective fractions of each genotype. No association was observed between mixed-versus single-genotype infections and the type of transplant received. Notably, gB2 was identified as the prevailing single genotype, while gB1-gB2 emerged as the predominant mixed genotypes in both transplantation settings. The prevalence of mixed genotypes was slightly higher in HSCT recipients compared to renal transplant recipients.

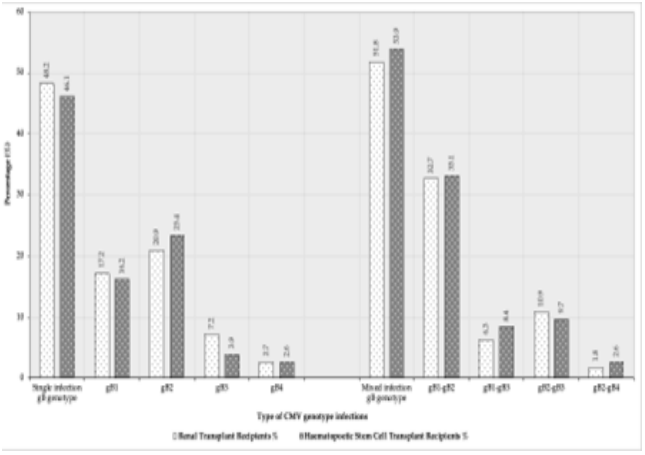

Figure 1: Distribution of glycoprotein B (gB) genotype in all patients and patients with mixed infection among renal and haematopoietic stem cell transplant recipients.

CMV gB genotypes and viral load

The comparison of CMV load was conducted among patients with different CMV gB genotypes (Figure 2). In renal transplant recipients, the median CMV load for each single genotype infection were as follows: gB1 showed the median viral load, 53,350 IU/mL (range: 250–185,700 IU/mL); followed by gB2, 52,540 IU/mL (range: 250–167,760 IU/mL); gB3, 33,205 IU/mL (range: 250– 62,500 IU/mL); and gB4, 18,750 IU/mL (range: 16,478– 20,250 IU/mL). In mixed genotype infections among renal transplant recipients, the median viral loads were as follows: gB1-gB2 had a median of 58,625 IU/mL (range: 250–224,550 IU/mL), gB1-gB3 had a median of 62,560 IU/mL (range: 18,680–189,500 IU/mL), gB2 and gB3 genotypes exhibited a median viral load of 54,754 IU/mL (range: 635–197,100 IU/mL), and gB2 and gB4 showed a median viral load of 147,720 IU/mL (range: 129,640–165,800 IU/mL).

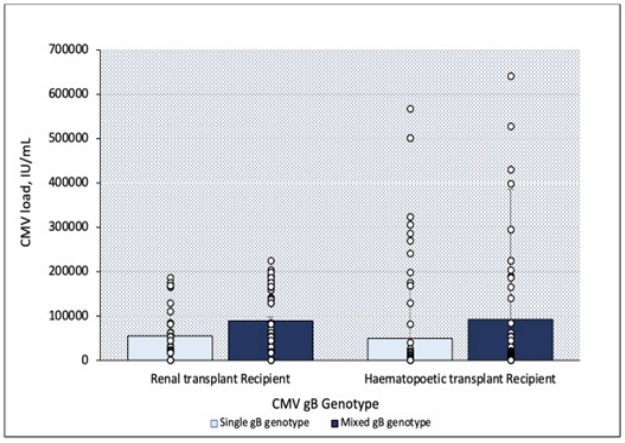

Figure 2: CMV viral load. The CMV viral loads in 110 plasma samples from renal transplant recipients and 154 haematopoietic stem cell transplant recipients were analysed. The samples were divided into two groups: single genotypes and mixed genotypes. The medians of each group are represented by lines. The study found a statistically significant difference in the median viral loads between the two groups (p<0.05) using Mann-Whitney U-Test.

A Mann-Whitney U-Test revealed a statistically significant difference between single and mixed genotype infections in the genotypes distribution. The single genotype infection group displayed lower values for viral load (Mdn = 52,200) compared to the mixed genotype infection group (Mdn = 62,500), U = 1,118, p = .019. A Kruskal-Wallis test indicated that there were no statistically significant difference in CMV genotypes (gB1, gB2, gB3, and gB4) among single genotype infections (H(3) = 5.11, p = .164) as well as mixed genotype infections (gB1-gB2, gB1-gB3, gB2-gB3 and gB2-gB4) (H(3) = 1.68, p = .641).

In HSCT recipients, the median CMV loads for single genotype infections were as follows: gB1 exhibited a median viral load of 494 IU/mL (range: 250–269,976), gB2 had a median of 1,195 IU/mL (range: 250–666,297), gB3 showed a median viral load of 693 IU/mL (range: 250–7,486), and gB4 had a median of 396 IU/mL (range: 250–497). In the context of mixed genotype infections among HSCT recipients, the median viral loads were observed as follows: gB1-gB2 had a median of 5,998 IU/mL (range: 250–792,667), gB1-gB3 exhibited a median viral load of 7,737 IU/mL (range: 495–83,696), mixed infections involving gB2 and gB3 genotypes displayed a median viral load of 5,095 IU/mL (range: 635–197,100), and mixed infections between gB2 and gB4 genotypes showed a median viral load of 1,330 IU/mL (range: 250–2,995).

A Mann-Whitney U-Test revealed a statistically significant distinction in genotypic distribution between single and mixed genotype infections. The single genotype infection group exhibited lower viral load values (Mdn = 682) compared to the mixed genotype infection group (Mdn = 682), with U = 1,725, and p < .001. Additionally, a Kruskal-Wallis test disclosed no statistically significant differences in CMV genotypes (gB1, gB2, gB3, and gB4) among both single genotype infections (H(3) = 6.32, p = .097) and mixed genotype infections (gB1-gB2, gB1-gB3, gB2-gB3, and gB2-gB4) (H(3) = 5.16, p = .161).

Relationship between CMV gB genotype, CMV disease and acute graft rejection

CMV disease was observed in 14 (11.4%) out of 264 renal and HSCT transplant recipients. The comparison of different gB in single and mixed genotype infections in developing CMV disease showed varying associations in two different transplant recipient populations as shown in Table IV. Among renal transplant recipients with CMV disease, 2.7% had a single-genotype infection, and 5.5% had mixed genotypes, whereas among those without CMV disease, 94.5% had a single-genotype infection, and 91.8% had mixed genotypes. The analysis revealed no statistically significant difference between the type of gB infection and the development of CMV disease in this group.

Table IV: Comparison of CMV Disease Incidence in Renal and HSCT Transplant Recipients with Single and Mixed gB Genotype Infections

Type of transplantation	Renal transplant recipients		p-value[a]
	With CMV disease	Without CMV disease	
Renal transplant recipients	n, (%)	n, (%)	
Single genotype	3 (2.7)	107 (94.5)	0.307
Mixed genotypes	6 (5.5)	104 (91.8)	
HSCT recipients			
Single genotype	1 (0.6)	153 (99.4)	0.176
Mixed genotypes	4 (2.6)	150 (97.4)	

[a]Chi-square test

Among HSCT recipients with CMV disease, 0.6% had a single-genotype infection, and 2.6% had mixed genotypes, whereas among those without CMV disease, 99.4% had a single-genotype infection, and 97.4%

had mixed genotypes. In HSCT recipients, mixed gB genotypes were found to have no statistically significant association with the development of CMV disease ($p<0.05$).

Acute graft rejection among renal transplant recipients was observed in 18 out of 110 renal transplant recipients, accounting for a rate of 16.4%. The rejection rates for each CMV gB group from the highest to lowest were as follows: 16.4% (3 out of 18 patients) for gB2, 11.1% (2 out of 18 patients) for gB1, 16.4% (3 out of 18 patients) for gB2, and 5.6% (1 out of 18 patient) for gB4. No statistically significant difference was found between the rates of graft rejection among patients infected with different CMV gB genotypes.

DISCUSSION

Cytomegalovirus (CMV) infection remains a significant concern in transplant recipients, with its impact varying based on the type of transplantation and the serostatus of both donor and recipient. Our study revealed important data on CMV serostatus and infection rates in both renal transplant and haematopoietic stem cell transplant (HSCT) recipients. The majority of recipients in both groups were seropositive (R+), with 89% of renal transplant cases and 84.4% of HSCT cases showing CMV seropositivity, regardless of donor status. Specifically, the D+/R+ combination was most prevalent, observed in 84.5% of renal transplant and 79.2% of HSCT recipients. Primary CMV infection, occurring in previously seronegative recipients (R-/D+), was less common but still significant, affecting 9.1% of renal transplant recipients and 11.7% of HSCT recipients. These findings underscore the importance of CMV management strategies in transplant recipients and set the stage for a deeper exploration of CMV genotypes and their clinical implications in these patient populations.

In this study, cytomegalovirus (CMV) genotypes distribution showed that gB2 was the most frequently identified single genotype, followed by gB1 while combination of gB1-gB2 was the predominant mixed gB genotypes in both renal and HSCT transplant recipients. However, studies conducted among renal transplant recipients in Sudanese and Northwest Indian populations reported a different genotype prevalence, where gB1 was identified as the most common, followed by gB2 and gB3 (17, 18). In a study cohort of HSCT patients in China, gB3 was found to be the predominant genotype, followed by gB1 and gB2. In contrast, gB1 and gB2 were the dominant genotypes in other ethnic groups (19). These discrepancies in genotype distribution could be due to different populations and geographical variation (19,25,26,28,29). Furthermore, the study revealed that CMV gB3 was associated with an elevated risk of CMV pneumonitis (19).

Recent clinical observations have shown that mixed strain gB genotypes of CMV are common and may lead to more severe disease progression (20). Mixed gB genotypes occur when genetically distant strains of CMV infect the same individual resulting in high within-host CMV nucleotide diversity especially in immunocompromised patients and have been associated with poor clinical outcomes (1,21,22). In HSCT recipients, we identified significant association between gB genotypes and CMV disease progression but not significant in renal transplant. This may suggest the presence of mixed gB genotypes vary in clinical implications depending on the type of transplantation and may vary according to underlying disease such there was a possible link between the gB subtype 2 and CMV retinitis in AIDS patients (48). Another study showed CMV gB2 genotype was associated with bad prognosis and outcome in AIDS, (49) while in Posner-Schlossman syndrome, CMV infection in the eye with gB genotype 3 is related to a more severe outcome (23). The underlying reasons could be multifaceted relating to the specific immune profiles of the recipient populations, the timing of CMV infection relative to transplantation, CMV tissue tropism and the immunosuppressive regimens employed in each setting (18, 23,24,25,26,27) which in turn affecting the virulence and clinical outcomes associated with CMV gB genotypes.

When comparing the CMV gB genotypes and their impact on viral load in renal transplant recipients and HSCT recipients, several key observations come to light. In renal transplant recipients, varying CMV load profiles were associated with different gB genotypes, with gB1 exhibiting the highest median viral load, followed by gB2, gB3, and gB4. Conversely, in HSCT recipients, gB2 displayed the highest median viral load among single genotype infections, followed by gB3, gB1, and gB4. Mann-Whitney U-Tests revealed a significant difference between single and mixed genotype infections in both groups, indicating that single genotype infections consistently had lower viral load values than mixed genotype infections. Furthermore, Kruskal-Wallis tests demonstrated no statistically significant differences in CMV genotypes within either group. This suggests that the influence of CMV gB genotypes on viral load remains consistent across renal transplant and HSCT recipients, emphasizing the importance of genotype distribution and its role in viral load variation across different transplant recipient categories.

This diverse distribution of CMV genotypes and their differential impact on viral loads lays the groundwork for understanding CMV's virulence, including its association with elevated viral loads, delayed viral clearance, and its interaction with the immune response. A study showed that there is no specific CMV glycoprotein B (gB) genotype conferring a specific CMV virulence. However, mixed gB genotypes were associated with higher viral loads and delayed viral clearance as seen in this study (30). CMV infection itself is associated

with the release of cytokines, chemokines, and growth factors, which can intensify immunosuppression and increase the risk of other opportunistic infections (31). This immunosuppression contributes to higher viral loads in the patient which may be exacerbated by mixed genotypes.

In renal transplant recipients, CMV viremia has been associated with increased mortality and allograft failure (32). A study found that subclinical CMV infection was independently associated with significant declines in glomerular filtration rate (GFR) during the first 2 years after transplantation (33). This suggests that CMV infection, even in the absence of clinical symptoms, can have a negative impact on renal function and contribute to higher viral loads. In HSCT recipients, CMV infection has been associated with a higher incidence of graft-versus-host disease (GVHD) (34). Hiwarkar et.al reported that CMV reactivation occurs in 40-70% of HSCT recipients who are seropositive or have a seropositive donor (35). The presence of GVHD and the immunosuppressive effects of HSCT may contribute to higher viral loads in these patients.

Genetic polymorphisms have also been implicated in the association between CMV genotypes and viral load. A study showed that patients carrying the CCR5 A/A genotype displayed episodes of active CMV infection with higher CMV viral load (36). Other studies have found that heterozygosity for toll-like receptor (TLR) 2 and TLR4 single nucleotide polymorphisms (SNPs) was associated with lower risk of CMV infection and lower levels of viremia (36). These genetic factors may contribute to the variability in viral load observed in renal and HSCT recipients.

In this study, CMV disease was observed in 8.2% of renal transplant recipients and in 3.2% of HSCT transplant recipients. It manifested in various forms, including colitis, hepatitis, esophagitis, gastrointestinal disease, diarrhoea, acute gastritis, and pneumonitis. The most common CMV syndrome observed in the patients was fever and leukopenia. The findings suggest that the impact of gB genotypes on CMV disease may differ based on the type of transplantation, with a significant association observed in HSCT recipients, while not significant in renal transplant recipients. Furthermore, the chances of progressing to CMV disease in both renal transplant and HSCT were higher in mixed compared to single gB genotype. In HSCT recipients, the mixed gB genotypes were significantly associated with the development of CMV disease.

Our findings align with other studies that suggest the presence of different CMV genotypes as a contributing factor to an increased risk of CMV disease and more severe clinical outcomes in mixed compared to single gB genotype infections. The impact of mixed infections of CMV genotypes on clinical outcomes in renal and HSCT transplantation can be attributed to several factors. Firstly, mixed infections have been associated with higher viral loads, which can contribute to more severe clinical manifestations and increased risk of complications (37,38). Higher viral loads are associated with increased risk of graft loss, patient mortality, and other complications (39,40,41). Secondly, mixed infections may result in a more diverse viral population, which can evade the immune response and lead to persistent or recurrent infections (44). This can contribute to prolonged viral replication and increased disease severity. Additionally, mixed infections may result in the co-infection of other herpesviruses, such as Epstein-Barr virus (EBV), which can further exacerbate the clinical outcomes (47). Co-infection with other pathogens can increase the risk of bacterial, fungal, and other pathogen infections, as well as graft-versus-host disease (GVHD) (41). Further research is needed to understand the underlying factors contributing to these differences and to refine the management of CMV infections in both populations.

Limitation

The study has several limitations. The sample size was relatively small, potentially limiting the generalizability of the findings. Being a single-centre study conducted in a tertiary hospital in Malaysia, the results may not be representative of other clinical settings or populations. The retrospective nature of the study and potential selection bias further impact the interpretation of the findings. Moreover, variations in laboratory techniques and the absence of longitudinal data restrict a comprehensive understanding of the dynamics of CMV genotypes. These limitations highlight the need larger, multicentre studies with standardized methods to address these gaps in future research.

CONCLUSION

In conclusion, our study showed the distribution of CMV genotypes, specifically gB genotypes, in renal and HSCT recipients. We found gB2 to be the most prevalent single genotype and gB1-gB2 as the predominant mixed gB genotypes. Mixed gB genotypes of CMV genotypes were associated with higher viral loads and more severe disease progression. Based on existing literature, it is well-established that the CMV genotype distribution can vary based on factors such as immune status, geographic location, patient population, and CMV tissue tropism. Genetic polymorphisms and immune responses play crucial roles in influencing the dynamic of CMV viral load, clinical outcomes in various medical conditions. Mixed gB genotypes contribute to diverse CMV strains, immune evasion, and co-infections with other pathogens. Understanding the impact of mixed gB genotypes is crucial for effective management of CMV infection in transplant recipients.

The data regarding the genetic diversity of gB in

this study population may shed a light on the varied genotypic distribution and its potential implications on CMV infection manifestations. The most promising CMV vaccine candidate tested to date is a soluble glycoprotein B (gB) subunit vaccine with MF59 adjuvant (gB/MF59), demonstrating 50% protection in multiple phase 2 clinical trials (50). Vaccine development should consider targeting on polyvalent gB genotypes, adapting to variations based on countries or underlying clinical and immune conditions, in order to elicit a strong immune response and confer protection against CMV disease.

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REFERENCES

1. Styczyński J.. Who Is the Patient At Risk Of CMV Recurrence: A Review Of The Current Scientific Evidence With A Focus On Hematopoietic Cell Transplantation. Infect Dis Ther 2017;7(1):1-16. https://doi.org/10.1007/s40121-017-0180-z
2. Wille PT, Wisner TW, Ryckman B, Johnson DC. Human Cytomegalovirus (HCMV) Glycoprotein gB Promotes Virus Entry In Trans Acting as the Viral Fusion Protein Rather than as a Receptor-Binding Protein. Hutt-Fletcher L, Nemerow G, editors. mBio. 2013 Jun 4;4(3).
3. Sarkar A, Das D, Ansari S, Chatterjee RP, Mishra L, Basu B, et al. Genotypes of glycoprotein B gene among the Indian symptomatic neonates with congenital CMV infection. BMC Pediatrics. 2019 Aug 22;19(1). https://doi.org/10.1186/s12887-019-1666-5
4. Girmenia C., Lazzarotto T., Bonifazi F., Patriarca F., Irrera G., Ciceri F.et al.. Assessment and Prevention Of Cytomegalovirus Infection In Allogeneic Hematopoietic Stem Cell Transplant And In Solid Organ Transplant: A Multidisciplinary Consensus Conference By The Italian Gitmo, Sito, And Amcli Societies. Clin Transplant 2019;33(10). https://doi.org/10.1111/ctr.13666
5. Jenks J., Nelson C., Roark H., Goodwin M., Pass R., Bernstein D.et al.. Antibody Binding To Native Cytomegalovirus Glycoprotein B Predicts Efficacy Of the Gb/mf59 Vaccine In Humans. Sci. Transl. Med. 2020;12(568). https://doi.org/10.1126/scitranslmed.abb3611
6. Shah A., Chen J., Fridell J.. Incidence and Outcomes Of Cytomegalovirus In Pancreas Transplantation With Steroid-free Immunosuppression. Clin Transplant 2015;29(12):1221-1229. https://doi.org/10.1111/ctr.12655
7. Jung S., Michel M., Stamminger T., Michel D.. Fast Breakthrough Of Resistant Cytomegalovirus During Secondary Letermovir Prophylaxis In a Hematopoietic Stem Cell Transplant Recipient. BMC Infect Dis 2019;19(1). https://doi.org/10.1186/s12879-019-4016-1
8. Kobashigawa J., Ross H., Bara C., Delgado J., Dengler T., Lehmkuhl H.et al.. Everolimus Is Associated With a Reduced Incidence Of Cytomegalovirus Infection Followingde Novocardiac Transplantation. Transpl Infect Dis 2012;15(2):150-162. https://doi.org/10.1111/tid.12007
9. Hu H., Peng Q., Chen K.. Clinical Features, Treatment Courses, and Distribution Of Cytomegalovirus Genotypes Among Thrombocytopenia Patients Aged Younger Than 12 Months. Am J Perinatol 2020;38(13):1403-1411. https://doi.org/10.1055/s-0040-1713001
10. Choo H., Cher W., Kwan Y., Fong W.. Risk Factors For Cytomegalovirus Disease In Systemic Lupus Erythematosus (Sle): a Systematic Review. Adv Rheumatol 2019;59(1). https://doi.org/10.1186/s42358-019-0055-y
11. Felipe C., Ferreira A., Paula M., Viana L., Cristelli M., Medina-Pestana J.et al.. Incidence and Risk Factors Associated With Cytomegalovirus Infection After The Treatment Of Acute Rejection During The First Year In Kidney Transplant Recipients Receiving Preemptive Therapy. Transpl Infect Dis 2019;21(6). https://doi.org/10.1111/tid.13106
12. Meeralam Y., Qurashi B., Masoudi A., Alhejaili T., Khayat M., Aljoaid A.et al.. Cytomegalovirus Colitis In a Patient With Ulcerative Colitis On Vedolizumab Monotherapy. Cureus 2023. https://doi.org/10.7759/cureus.35473
13. Pardieck I., Duikeren S., Veerkamp D., Brasem D., Redeker A., Bergen J.et al.. Dominant Antiviral Cd8+ T Cell Responses Empower Prophylactic Antibody-eliciting Vaccines Against Cytomegalovirus. Front. Immunol. 2022;13. https://doi.org/10.3389/fimmu.2022.680559
14. Manuel O, Esberg A, Pang X, Rollag H, Emery Vincent C, Preiksaitis J, et al. Impact of Genetic Polymorphisms in Cytomegalovirus Glycoprotein B on Outcomes in Solid-Organ Transplant Recipients with Cytomegalovirus Disease. Clinical Infectious Diseases. 2009 Oct 15;49(8):1160–6.
15. Hashim A., Altayb H., Saeed S., Abdalla W., Omer A.. Molecular Detection and Glycoprotein B (Ul55) Genotyping Of Cytomegalovirus Among Sudanese Renal Transplant Recipients. BioMed Research International 2022;2022:1-9. https://doi.org/10.1155/2022/5403694
16. Dieamant D., Bonon S., Aranha F., Duarte G.,

Fernandes V., Souza C.et al.. Cytomegalovirus (Cmv) Gb3 Genotype Is Associated With Acute Gvhd and CMV Disease In Allogeneic Hematopoietic Stem Cell Transplant Recipients (Hsct). Blood 2011;118(21):1958-1958. https://doi.org/10.1182/blood.v118.21.1958.1958

17. Hashim A., Altayb H., Saeed S., Abdalla W., Omer A.. Molecular Detection and Glycoprotein B (Ul55) Genotyping Of Cytomegalovirus Among Sudanese Renal Transplant Recipients. BioMed Research International 2022;2022:1-9. https://doi.org/10.1155/2022/5403694
18. Khalafkhany D., Makhdoomi K., Afshari A., Motazakker M.. Prevalence and Genotype Distribution Of Cytomegalovirus Ul55 Sequence In Renal Transplant Recipients In North West Of Iran. J. Med. Virol. 2016;88(9):1622-1627. https://doi.org/10.1002/jmv.24509
19. Wu X., Wang Y., Xu Y., Wu D., Sun A., Zhu Z.et al.. Cytomegalovirus Glycoprotein B Genotype In Hematopoietic Stem Cell Transplant Patients From China. Biology of Blood and Marrow Transplantation 2010;16(5):647-652. https://doi.org/10.1016/j.bbmt.2009.12.001
20. Wang H., Valencia S., Pfeifer S., Jensen J., Kowalik T., Permar S.. Common Polymorphisms In the Glycoproteins Of Human Cytomegalovirus And Associated Strain-specific Immunity. Viruses 2021;13(6):1106. https://doi.org/10.3390/v13061106
21. Cudini J., Roy S., Houldcroft C., Bryant J., Depledge D., Tutill H.et al.. Human Cytomegalovirus Haplotype Reconstruction Reveals High Diversity Due To Superinfection and Evidence Of Within-host Recombination. Proc. Natl. Acad. Sci. U.S.A. 2019;116(12):5693-5698. https://doi.org/10.1073/pnas.1818130116
22. Pati S., Pinninti S., Novak Z., Chowdhury N., Patro R., Fowler K.et al.. Genotypic Diversity and Mixed Infection In Newborn Disease And Hearing Loss In Congenital Cytomegalovirus Infection. The Pediatric Infectious Disease Journal 2013;32(10):1050-1054. https://doi.org/10.1097/inf.0b013e31829bb0b9
23. Wang Z., Sheng Q., Fan X., Sun X.. Polymorphisms Of the Cytomegalovirus Glycoprotein B Genotype In Patients With Posner-schlossman Syndrome. Br J Ophthalmol 2021;106(9):1240-1244. https://doi.org/10.1136/bjophthalmol-2020-318284
24. Hu H., Peng Q., Chen K.. Clinical Features, Treatment Courses, and Distribution Of Cytomegalovirus Genotypes Among Thrombocytopenia Patients Aged Younger Than 12 Months. Am J Perinatol 2020;38(13):1403-1411. https://doi.org/10.1055/s-0040-1713001
25. Paradowska E., Studzińska M., Suski P., Kasztelewicz B., Wiśniewska-Ligier M., Zawilińska B.et al.. Human Cytomegalovirus Ul55, Ul144, and Us28 Genotype Distribution In Infants Infected Congenitally Or Postnatally. J. Med. Virol. 2015;87(10):1737-1748. https://doi.org/10.1002/jmv.24222
26. Cella E., Ritta M., Ciccozzi M., Cavallo R., Perno C., Costa C.. Cytomegalovirus Glycoprotein B Genotype Distribution In Italian Transplant Patients. Intervirology 2017;60(4):165-170. https://doi.org/10.1159/000486593
27. Berg C., Rosenkilde M., Benfield T., Nielsen L., Sundelin T., Lьttichau H.. The Frequency Of Cytomegalovirus Non-elr Ul146 Genotypes In Neonates With Congenital CMV Disease Is Comparable To Strains In the Background Population. BMC Infect Dis 2021;21(1). https://doi.org/10.1186/s12879-021-06076-w
28. White J., Patel E., Abraham A., Grabowski M., Arav-Boger R., Avery R.et al.. Prevalence, Magnitude, and Genotype Distribution Of Urinary Cytomegalovirus (Cmv) Shedding Among Cmv-seropositive Children And Adolescents In The United States. Open Forum Infectious Diseases 2019;6(7). https://doi.org/10.1093/ofid/ofz272
29. Nijman J., Mandemaker F., Verboon-Maciolek M., Aitken S., Loon A., Vries L.et al.. Genotype Distribution, Viral Load and Clinical Characteristics Of Infants With Postnatal Or Congenital Cytomegalovirus Infection. PLoS ONE 2014;9(9):e108018. https://doi.org/10.1371/journal.pone.0108018
30. Manuel O., Esberg A., Pang X., Rollag H., Emery V., Preiksaitis J.et al.. Impact Of Genetic Polymorphisms In Cytomegalovirus Glycoprotein B On Outcomes In Solid-organ Transplant Recipients With Cytomegalovirus Disease. CLIN INFECT DIS 2009;49(8):1160-1166. https://doi.org/10.1086/605633
31. Hashim A., Altayb H., Saeed S., Abdalla W., Omer A.. Molecular Detection and Glycoprotein B (Ul55) Genotyping Of Cytomegalovirus Among Sudanese Renal Transplant Recipients. BioMed Research International 2022;2022:1-9. https://doi.org/10.1155/2022/5403694
32. Selvey L., Lim W., Boan P., Swaminathan R., Slimings C., Harrison A.et al.. Cytomegalovirus Viraemia and Mortality In Renal Transplant Recipients In The Era Of Antiviral Prophylaxis. Lessons From The Western Australian Experience. BMC Infect Dis 2017;17(1). https://doi.org/10.1186/s12879-017-2599-y
33. Smith J., Corey L., Bittner R., Finn L., Healey P., Davis C.et al.. Subclinical Viremia Increases Risk For Chronic Allograft Injury In Pediatric Renal Transplantation. Journal of the American Society of Nephrology 2010;21(9):1579-1586. https://doi.org/10.1681/asn.2009111188
34. El Helou G, Razonable R. Letermovir for the prevention of cytomegalovirus infection and disease in transplant recipients: an evidence-based review. Infection and Drug Resistance. 2019

Jun;Volume 12:1481–91. https://doi.org/10.2147/idr.s180908
35. Hiwarkar P., Gaspar H., Gilmour K., Jagani M., Chiesa R., Bennett-Rees N.et al.. Impact Of Viral Reactivations In the Era Of Pre-emptive Antiviral Drug Therapy Following Allogeneic Haematopoietic Sct In Paediatric Recipients. Bone Marrow Transplant 2012;48(6):803-808. https://doi.org/10.1038/bmt.2012.221
36. Vallejo M., Muñiz P., Kwon M., Solán L., Bailén R., Carbonell D.et al.. Risk Prediction Of CMV Reactivation After Allogeneic Stem Cell Transplantation Using Five Non-hla Immunogenetic Polymorphisms. Ann Hematol 2022;101(7):1567-1576. https://doi.org/10.1007/s00277-022-04841-8
37. Manuel O., Åsberg A., Pang X., Rollag H., Emery V., Preiksaitis J.et al.. Impact Of Genetic Polymorphisms In Cytomegalovirus Glycoprotein B On Outcomes In Solid-organ Transplant Recipients With Cytomegalovirus Disease. CLIN INFECT DIS 2009;49(8):1160-1166. https://doi.org/10.1086/605633
38. Huang C., Cheng Y., Hwang Y., Kang E., Hsiao C.. Cytomegalovirus Glycoprotein B Genotype In Patients With Anterior Segment Infection. IJMS 2023;24(7):6304. https://doi.org/10.3390/ijms24076304
39. Zou J.. Clinical Manifestations and Outcomes Of Renal Transplantation Patients With Pneumocystis Jirovecii Pneumonia And Cytomegalovirus Co-infection. Front. Med. 2022;9. https://doi.org/10.3389/fmed.2022.860644
40. Teschner D., Knop J., Piehl C., Junker S., Witzke O.. Cytomegalovirus Infection and Rehospitalization Rates After Allogeneic Hematopoietic Stem Cell And Solid Organ Transplantation: A Retrospective Cohort Study Using German Claims Data. Infection 2022;50(6):1543-1555. https://doi.org/10.1007/s15010-022-01847-2
41. Chen P., Du Z., Qiu S., Wang H., Zhang J., Liu D.. Current Research Status Of Cytomegalovirus Infection After Hematopoietic Stem Cell Transplantation: a Bibliometric Analysis. Ann Palliat Med 2021;10(9):9614-9626. https://doi.org/10.21037/apm-21-2301
42. Yu X., Shang Q., Liu X., He M., Pei X., Mo X.et al.. Donor Nkg2c Homozygosity Contributes To CMV Clearance After Haploidentical Transplantation. Jci Insight 2022;7(3). https://doi.org/10.1172/jci.insight.149120
43. Ljungman P., Hakki M., Boeckh M.. Cytomegalovirus In Hematopoietic Stem Cell Transplant Recipients. Hematology/Oncology Clinics of North America 2011;25(1):151-169. https://doi.org/10.1016/j.hoc.2010.11.011
44. Huang C., Cheng Y., Hwang Y., Kang E., Hsiao C.. Cytomegalovirus Glycoprotein B Genotype In Patients With Anterior Segment Infection. IJMS 2023;24(7):6304. https://doi.org/10.3390/ijms24076304
45. Liu S., Bos N., Verschuuren E., Baarle D., Westra J.. Biological Characteristics Of Hla-g and Its Role In Solid Organ Transplantation. Front. Immunol. 2022;13. https://doi.org/10.3389/fimmu.2022.902093
46. Mostafa M., Fathy A., Elwasify M., Abdelsalam M.. Analysis Of Selected Polymorphisms In Foxp3 Gene In a Cohort Of Egyptian Patients With Schizophrenia. J Genet Eng Biotechnol 2022;20(1). https://doi.org/10.1186/s43141-022-00371-y
47. Barani R., Mani M., Sarangan G., Soundararajan P., Palani G., Srikanth P.. CMV Genotyping Using Different Samples In Post Renal Transplant Recipients With Cmv Disease. Can J Biotech 2017;1(Special Issue):93-93. https://doi.org/10.24870/cjb.2017-a80
48. Vogel JU, Otte J, Koch F, H. Gьmbel, Hans Wilhelm Doerr, Jindrich Cinatl. Role of human cytomegalovirus genotype polymorphisms in AIDS patients with cytomegalovirus retinitis. 2012 Jun 6;202(1):37–47. https://doi.org/10.1007/s00430-012-0244-3
49. Aldo Albuquerque Cunha, Vitor Coronel Aquino, Viviane Casagrande Mariguela, Maurнcio Lacerda Nogueira, Luiz. Evaluation of glycoprotein B genotypes and load of CMV infecting blood leukocytes on prognosis of AIDS patients. 2011 Apr 1;53(2):82–8. https://doi.org/10.1007/s00430-012-0244-3
50. Jenks, Jennifer A, et al. Antibody Binding to Native Cytomegalovirus Glycoprotein B Predicts Efficacy of the GB/MF59 Vaccine in Humans. Vol. 12, no. 568, 4 Nov. 2020, https://doi.org/10.1126/scitranslmed.abb3611. Accessed 6 Aug. 2023.