The Incidence of Cytomegalovirus Glycoprotein B Genotypes in Kidney Transplant Recipients in Iran

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ABSTRACT

Background: Cytomegalovirus (CMV) is the most common opportunistic viral infection in kidney transplant recipients. CMV classification is usually based on its glycoprotein B (gB) genotypes, which divides the virus into 4 strains (gB1–4).

Objective: To determine the incidence of CMV genotypes in Iran and their relation to various clinical factors.

Methods: We studied 80 renal transplant recipients admitted to our transplant referral center between 2014 and 2015. All of the studied patients were monitored every 1–2 weeks for CMV infection by immunofluorescence method. There were 34 CMV-infected patients whose sera were studied with sequencing technique to identify the 4 CMV genotypes. All patients were followed up to 6 months after transplantation.

Results: gB1 was the most common genotype (35.3%); it was followed by gB3 and gB4 (each with 17.6 %), gB2, and mixed gB1,3 and gB1,2 (each with 14.7%). Age (p=0.037), time of infection after transplantation (p=0.011), and biopsy-proven rejection (p=0.012) were associated with CMV genotype. After adjusting for covariates, significant associations were found between genotype gB1 and family relationship (p=0.047) as well as HLA mismatch (p=0.014); genotype gB3 and family relationship (p=0.011); and genotype gB4 and age (p=0.019).

Conclusion: The most common CMV gB genotype in CMV-infected kidney transplant recipients in Iran was gB1. We recommend considering related therapeutic applications in the management of such patients.

KEYWORDS: CMV infection; Glycoprotein B; Genotype; Renal transplantation

INTRODUCTION

Cytomegalovirus (CMV) is a DNA virus with an estimated size of 200 nanometers and belongs to Herpes virus family [1]. CMV infection continues to be a major clinical problem after solid organ transplantation with a significant morbidity and mortality. It causes symptomatic disease in 35% and death in 2% of renal transplant recipients [2]. Although gancyclovir and related drugs reduce almost 50%–70% of CMV disease incidence as well as its mortality [3, 4], the toxicity associated with the use of currently available antiviral agents remains a significant problem [5]. Previous studies have shown that the immune response against CMV may strongly be dependent on the virus strain [6] and that reinfection with other strains may be completely different clinically from the relapse of the first

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one [7]. Moreover, the presence of only one CMV strain compared to several ones in the first year after transplantation is associated with different clinical outcomes. For example, it has been demonstrated that mixed CMV-strain infection in organ transplant recipients could be associated with a higher transplant rejection rate, delayed virus clearance from the blood and faster disease development [8].

The classification of CMV strains is usually done based on the virus glycoprotein B (gB) genotype [9], which could be done by either sequencing or restriction fragment length polymorphism (RFLP). Although not suitable for fast-screening large populations, such methods would be valuable in infected cases in whom different strains may have different pathogenicity needing different approaches and treatment [10].

In line with a previous study conducted in a local population of renal transplant recipients in Northwest of Iran [11], we designed this study in a general population of Iranian people to evaluate CMV gB distribution in CMV-infected renal transplant recipients. We also correlated different demographic as well as clinical characteristics of the patients to the studied genotypes.

MATERIALS AND METHODS

Out of 400 kidneys transplant recipients, 80 were randomly enrolled into our cross-sectional study conducted between 2014 and 2015 in Baqiyatallah Hospital, a referral center for kidney transplant from all over Iran. The sample size was calculated based on an estimated incidence of CMV infection in kidney transplant patients of 80%, and 5% type I error, and 8% accuracy, using Cochran's formula. All patients had a living donor and followed for a period of six months. The induction as well as maintenance immunosuppression protocol for all recipients included therapeutic adjusted doses of calcineurin inhibitors, mycophenolate mofetil, and steroids. All patients were followed for six months on a monthly basis and were studied for the type of prescribed immunosuppressive drugs (cyclosporine A or tacrolimus), CMV infection defined according to the standard criteria, and biopsy-proven acute rejection. This study was approved by the local Ethics Committee. Informed consent was obtained from all participants.

All of the studied patients were monitored every 1–2 weeks for active CMV infection using antigenemia (AGM) assay. Using the Light Diagnostics CMV phosphoprotein (pp)65 Antigenemia Immunofluorescence Assay (IFA), we utilized an indirect immunofluorescence technique to identify the lower matrix protein pp65 of human CMV in cytospin preparations of peripheral blood leukocytes (Merck Millipore LIGHT DIAGNOSTICS CMV pp65 Antigenemia IFA kit).

Briefly, ethanol diamine tetra acetic acid (EDTA)-treated blood samples were fractionated by erythrocyte lyses. Granulocytes were then centrifuged to prepare cytospin slides (2×10^5 granulocytes per slide). After air-drying and fixing the slides in formaldehyde, they were immunostained using sufficient CMV pp65 monoclonal antibody to detect the CMV lower matrix phosphoprotein (pp65), an early antigen in virus replication, which is abundantly present in antigen-positive polymorphonuclear cells. Finally, the samples were examined with a fluorescence microscope under a magnification of 200–400×, for cells exhibiting the apple green fluorescence of fluorescent iso-thio cyanate (FITC).

CMV gB genotyping was done according to a method described elsewhere [12]. In brief, DNA extracted from 200 mL whole peripheral blood with the DNA blood and tissue extraction kit (Qiagen, Chatsworth, CA, USA) was used as template. Then, polymerase chain reaction (PCR) was performed using primers gB1319 (5'-TGGAACTGGAACGTTTGGC-3') and gB1604 (5'-GAAACGCGCGGCAATCGG-3') [13], which amplify a region of unique long (UL)55 that encodes a variable part of gp55 yielding a 293–296 bp target, depending on the gB genotype. Each reaction was done in a final volume of 100 µL and contained 10 mM Tris-HCl (pH 8.3), 50

Table 1: The frequency, mean and SD of studied variables stratified by gB CMV genotypes. Values are either mean±SD or n (%).

Variable	gB					
	1	2	3	4	mix	p value
Sex						
Female	5 (42)	3 (60)	3 (50)	3 (50)	3 (60)	0.968
Male	7 (58)	2 (40)	3 (50)	3 (50)	2 (40)	
Mean±SD Age (yrs)	47.5±7.8	39.4±5.6	49.3±11.1	34.1±8.1	43.6±13.3	0.037
Mean±SD time of infection (months)	4.6±1.4	2.6±2.1	3.6±1.6	2.3±1.0	1.8±1.7	0.011
Rejection						
No	11 (92)	2 (40)	5 (83)	2 (33)	1 (20)	0.012
Yes	1 (8)	3 (60)	1 (17)	4 (67)	4 (80)	
Family relation between recipient and donor						
No	8 (67)	4 (80)	2 (33)	5 (83)	3 (60)	0.432
Yes	4 (33)	1 (20)	4 (67)	1 (17)	2 (40)	

mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 µM of each dNTP, 0.25 µM of each primer, 2.5 units of Taq polymerase (Amplitaq; Perkin Elmer, Markham, Ont, Canada) and 10–30 µL of template DNA. Presence of the correct amplicon was verified by gel electrophoresis on 2% agarose.

Prior to be restricted and sequenced, the PCR products were also gel purified using the QIAquick gel extraction protocol (Qiagen Inc., Mississauga, Ont, Canada). The purified amplicon from each isolate was separately digested with HinfI and RsaI. Without running on gel, some of the isolates were sequenced to confirm that the restriction digests were producing reliable results. The sequences obtained in this study were aligned (Vector NTI 5.5) with those that were previously sequenced and typed by Chou and Dennison (Genbank accession numbers: M60928, M60930, M60931, and M60933).

Statistical Analysis

The results were expressed as mean±SD. Normality test was performed for the examined variables. The groups were compared by χ^2 test and ANOVA. In addition, we applied multiple logistic regression model with conditional backward method to adjust the effect of independent variables on CMV genotypes. A p value <0.05 was considered statistically significant. All analyses were made by the SPSS 18 package.

RESULTS

CMV Infection Incidence

From all 80 renal transplant recipients examined, 34 (48%) showed CMV infection during six months. The most infection rate with CMV happened during the first and second months (11% and 9%, respectively).

Distribution of CMV gB Genotypes

Genotyping was examined in 34 viremic recipients. The most common CMV gB genotype was gB1 (35.3%) followed by gB3 and gB4 (each with 17.6%), gB2 and mixed gB1,3 and gB1,2 (each with 14.7%) (Table 1).

Evaluating the frequency of different variables according to the CMV gB genotypes, we found that rejection showed a significant relationship to gB strains (p=0.012) (Table 1); the most transplant rejection happened in gB mix (80%); the least was in those with gB1 (8.3%) . The relationship between CMV gB genotype and age (p=0.037) as well as the time of genotyping after transplantation (p=0.011) was also statistically significant (Table 1).

After adjusting for covariates, the correlation between sex, age, family relation between the recipient and donor, and HLA mismatch, and different CMV gB genotypes, was studied. We found that some variables had a significant relationship to some gB genotypes (Table

Table 2: Association of variables with CMV genotypes after adjusting for covariates

Genotype	Variable	p value	OR
gB1	Sex (male)	0.532	0.675
	Age	0.112	1.049
	Family relation (yes)	0.047	4.126
	HLA mismatch	0.014	2.240
gB2	Sex (male)	0.191	0.268
	Age	0.300	0.940
	Family relation (yes)	0.692	1.657
	HLA mismatch	0.125	2.093
gB3	Sex (male)	0.245	0.314
	Age	0.115	1.069
	Family relation (yes)	0.011	17.087
	HLA mismatch	0.193	1.957
gB4	Sex (male)	0.085	0.150
	Age	0.019	0.838
	Family relation (yes)	0.895	1.177
	HLA mismatch	0.070	2.234
Mixed	Sex (male)	0.348	0.414
	Age	0.972	0.998
	Family relation (yes)	0.285	3.078
	HLA mismatch	0.454	1.410

2). According to this regression model, family relation (p=0.047) and HLA mismatch (p=0.014) were two independent predictors of gB1 infection; family relation was an independent predictor of gB3 infection (p=0.011); age was an independent predictor of gB4 infection (p=0.019) (Table 2).

DISCUSSION

Considering that the immune response as well as clinical outcome of CMV infection after solid organ transplantation may strongly be dependent on the virus strain [6], we studied the distribution of CMV strains in Iranian kidney transplant recipients, based on virus glycoprotein B (gB) genotype. We also evaluated the association between different CMV genotype strains and a number of factors.

Similar to our findings, other studies on different populations such as those residing in northern Iran [11], Turkey [14], and Kuwait [15] showed that gB1 was the most prevalent CMV genotype (26.5%, 32.3%, and 59%, respectively). This has also been shown in the study of Pang and colleagues on 121 plasma specimens from 47 solid organ transplant recipients in whom the next most common genotypes were also gB2, mixed gB, gB3 and gB4 [16]. However, there were differences. For example, in Kuwait, gB2 and gB3 (29% and 10%, respectively) were the most prevalent strains too [15] that was in contrast with our findgins most probably due to racial differences.

The association between different CMV genotypes and different clinical characteristics of the transplant recipients has also been identified in other studies. Madi, *et al*, demonstrated that gB1 genotype is significantly associated with development of fever, leukopenia and severe CMV disease compared with other gB genotypes [15]. In a study by Rosen and colleagues on 53 CMV-infected liver transplant recipients, it was shown that gB1 genotype is significantly associated with a higher number of acute rejection episodes but not with the rejection severity [17]. Correa revealed that gB4 is the only genotype associated with clinical features of sepsis-like syndrome in newborns [18]. In contrast, Sarcinella, *et al*, in a study on 58 liver transplant recipients with CMV infection showed that gB genotype does not correlate with peak CMV viral load, development of CMV disease, and acute rejection [12].

Age, time of genotyping after transplantation, and biopsy-proven rejection showed a significant correlation with gB strains in our study. Although CMV infection was not an independent risk factor for rejection, CMV gB genotype was associated with transplant rejection in CMV-infected patients. After adjusting for covariates, family relation and HLA mismatch were associated with gB1, family relation was associated with gB3, and age was associated with gB4.

Further studies are needed to clarify the role of CMV gB genotypes in the clinical manifestations in transplant recipients and also to reveal the factors that influence the CMV gB genotype.

In conclusion, the most common CMV gB genotype in CMV-infected kidney transplants of Iran was gB1. Physicians should consider he related factors such as rejection in the management of kidney transplant recipients.

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