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Cytomegalovirus Infection Among Iranian Kidney Graft Recipients

Z.R. Khameneh, N. Sepehrvand, and T. Aghazadeh

ABSTRACT

Background. Cytomegalovirus (CMV) infection is one of the most common infectious problems following kidney transplantation. In this study we sought to investigate CMV infection in the setting of renal transplant recipients in Urmia, Iran, using polymerase chain reaction (PCR) detection.

Methods. Ninety-six randomly selected renal transplant recipient were enrolled in a cross-sectional study. Blood sampling via venipuncture, yielded sera investigated for anti-CMV IgM. Seropositive as well as 14 randomly selected seronegative cases were investigated with PCR assays.

Results. Thirty-three patients (34.3%) were seropositive for anti-CMV IgM; 3 (3.1%) borderline, and 60 (62.5%) seronegative. Considering borderline anti-CMV IgM levels as seropositive, 37.5% were seropositive for anti-CMV IgM. Among the 36 seropositive cases, a CMV infection was confirmed in 19 (52.7%) using PCR. Age ($P = .40$), educational status ($P = .77$), history of pretransplantation dialysis ($P = .52$), prior blood transfusion ($P = .52$), and immunosuppressive regimen were not significantly different among positive versus negative CMV PCR recipients.

Conclusions. The seroprevalence of CMV infection was high among renal transplant recipients of Urmia, Iran, as confirmed by PCR study.

Cytomegalovirus (CMV), a β-herpesvirus, is endemic in all regions of the world.[1] CMV infection among healthy children or adults is usually asymptomatic, but causes significant morbidity and mortality among transplant recipients.[2] Because of their immunocompromised nature due to immunosuppressant medications, transplant recipients, are more susceptible to be infected with viral and bacterial agents.[3–5] Several studies have demonstrated that CMV infection increases graft loss and is associated with death resulted from all causes.[6]

Cytomegalovirus influences the condition of immunocompromised patients in 2 ways: first, direct effects (viral syndrome, pneumonia, meningoencephalitis, and gastrointestinal tract involvement); and second, indirect (immunomodulatory) effects.[7–9]

Most studies of graft recipients have used the antigenemia (enzyme-linked immunosorbent assay [ELISA] method) to document the presence of CMV infection, but now detection by polymerase chain reaction (PCR) is the method of choice. In the present study, we sought to investigate CMV infection among a representative sample of renal transplant recipients in Urmia, Iran.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted with the approval of our Scientific and Ethical Review Board. One hundred adult first kidney transplant recipients from a living or deceased donor were included in this study. One of every 5 recipients referred to our Department over a 3-month period was selected randomly, excluding multiorgan or second kidney transplantations and those unwilling to participate (n = 4). Finally we enrolled 96 recipients. Who provided informed consent before participation. Via venipuncture we obtained a 5-cc blood sample for serologic study. After centrifugation sera separated without delay were stored at −20°C.

ELISA

All sera were investigated for anti-CMV IgM using ELISA. The presence of anti-CMV IgM antibody was considered to be evidence of a current CMV infection.

From the Urmia University of Medical Sciences, Urmia, Iran.

Address reprint requests to Dr Nariman Sepehrvand, Students' Research Committee, Urmia University of Medical Sciences, Resalat Ave., Djahad Sq., Urmia, Iran. E-mail: nariman256@gmail.com

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PCR

CMV DNA determinations in serum samples were performed using primer pairs for CMV DNA for amplification. The forward and reverse primers were 5′-CGC GGT TAG AAT CGA GGA CCA TAG-3′ and 5′-TTA CCC AGC CTC ATC TCC GTA CCT-3′, respectively.

The reaction buffer contained 50 mmol/L KCl; 10 mL Tris-HCl, pH 8.3; 1.5 mmol/L $MgCl_2$; 0.01% gelatin as well as 200 mmol/L of each of the 4 deoxynucleotide triphosphates (dATP, dCTP, dTTP, and dGTP) plus 2.4 μmol/L primer, distilled water, and sample (5 μL), totaling 100 μl. After the reaction mixture was overlain with 100 μL of mineral oil, the tubes were placed in a boiling water bath for 7 minutes. We added 0.4 μL (2 U) Thermus aquaticus (Taq) polymerase. The amplification reaction was performed in a DNA thermal cycler (Bio-ER, Japan). The samples were heated to 95°C for 180 seconds to denature DNA, cooled to 62°C for 40 seconds for annealing, and then heated to 72°C for 40 seconds for extension. In the final cycle the samples were heated to 93°C for 40 seconds, then cooled to 61°C for 40 seconds and heated again to 72°C for 40 seconds. The final cycle was repeated 35 times; 8 μL of the amplified product was detected by direct analysis using 2 μL dye on 2% agarose minigels. A 222-base-pair band was seen when samples were amplified with primer (Fig 1).

Statistical Analysis

Data were collected regarding the following variables: age, sex, education, marital status, end-stage renal disease etiology and duration, hemodialysis history, prior blood transfusions, and immunosuppressive therapy.

All data were analyzed with SPSS software v16 (Chicago, IL). Descriptive statistics reported as mean ± SD for continuous variables and as frequency (%) for dichotomous variables. To evaluate relationships between factors we performed chi-square analysis. Quantitative variables were compared using independent-sample *t* tests. *P* values of <.05 were considered to be statistically significant.

RESULTS

Ninety-six renal transplant recipients were selected randomly among kidney transplant recipients from 1991 to 2010.

The overall mean age of the patients was 35.9 ± 14.4 years (range, 6–65). Fifteen patients (15.6%) were ≤19, 39 (40.6%) 20–39, and 42 subjects (43.8%) ≥40 years old. Sixty-five patients (67.7%) were men. Sixty-four (66.7%) were married. Twenty patients (20.8%) were illiterate; 23 (24%) studied to the elementary level; 10 (10.4%), to guidance school; 10 (10.4%), to high school; 32 (33.3%) had a diploma, and only 1 (1%) had an academic degree.

Their etiologies of renal failure were glomerulonephritis (n = 34; 35.4%), hypertension (n = 31, 32.3%), Polycystic kidney disease (n = 13; 13.5%), nephrolithiasis (n = 2), and focal-segmental glomerulosclerosis, diabetes mellitus, Alport syndrome, neurogenic bladder, and urinary infection each in only 1 (1.1%) participant.

Sixty-eight patients (70.8%) underwent hemodialysis before renal transplantation, but 28 (29.2%) had no history of HD prior to transplantation. Only 37 subjects (38.5%) had a history of blood transfusion. Sixty-six subjects (68.8%) received an immunosuppressive regimen including cyclosporine, mycophenolate mofetil, and prednisolone, and 30 cyclosporine, azathioprine, and prednisolone.

By ELISA, 33 patients (34.3%) were seropositive for anti-CMV IgM, 3 (3.1%) were borderline, and 60 (62.5%) seronegative. Considering borderline anti-CMV IgM levels as seropositive, we obtained 37.5% seropositives, all of whom were included in the PCR study. Additionally, 14/60 seronegative cases were selected randomly to undergo the PCR assay.

Among seropositive cases, 19 showed CMV infection by PCR and 17 were negative. Only 1 seronegative case showed a CMV infection by PCR evaluation. All 3 patients with borderline ranges of anti-CMV IgM were negative by PCR. The characteristics of positive or negative CMV PCR patients are compared in Table 1.

Pearson chi-square analysis revealed a significant relationship between the presence of CMV DNA in PCR and seropositivity for anti-CMV IgM ($P = .003$). Table 1 presents age ($P = .40$), education ($P = .77$), pretransplantation dialysis ($P = .52$), prior blood transfusion ($P = .52$), and immunosuppressive regimen to not be significantly different among positive versus negative CMV PCR recipients. Only sex was different ($P = .042$): The positive cases were mostly women, and most negative cases (73.3%) were men.

The same analysis was performed for ELISA seropositive and seronegative groups, showing none of above characteristics—age, sex, educational status, pretransplantation dialysis, history of blood transfusion—to be significantly different between seropositive and seronegative groups (Table 1).

As presented in Table 2, the sensitivity of the ELISA method to investigate anti-CMV IgM was 95% (95% CI, 73%–99.7%) with a specificity of 43.3% (95% CI, 25.9%–62.3%).

DISCUSSION

CMV is the main cause of infectious complications after kidney transplantation.[10] The disease is symptomatic in <20% of graft recipients, and is responsible for 18% of posttransplantation infections.[11] It is an important risk factor for graft rejection and recipient morbidity and mortality.[12] Because of the immunosuppressed nature of transplant recipients, and the probability of exposure to the virus via its transmission routes of blood transfusion and organs, this population is at the highest risk for a primary CMV infection or reactivation of a latent infection.

According to the present results, the seroprevalence of CMV infection (anti-CMV IgM) was 34.3% without considering borderline cases, and 37.5% including borderline cases. By further PCR study the infection was confirmed in 19 seropositive patients (52.7%). Dacunha et al reported the prevalence of CMV infection in renal transplant recipients to be 25.5%.[13] Cavdar et al observed 24.1% of recipients to develop CMV disease.[12] In another Iranian study on renal transplant recipients, by Tarabadi et al,

Table 1. Patients' Characteristics Among Two Groups With Positive or Negative PCR Results

Characteristic	CMV Seropositive	CMV Seronegative	*P* Value	CMV PCR Positive	CMV PCR Negative	*P* Value
Age (mean ± SD), y	38.5 ± 15.2	34.4 ± 13.7	.17	40.2 ± 14.4	36.5 ± 16.0	.40
Age						
<20	4 (11.1%)	11 (18.3%)	.34	2 (10%)	6 (20%)	.63
20–40	13 (36.1%)	26 (43.3%)		7 (35%)	9 (30%)	
>40	19 (52.8%)	23 (38.3%)		11 (55%)	15 (50%)	
Sex						
Female	14 (38.9%)	17 (28.3%)	.28	11 (55%)	8 (26.7%)	.042*
Male	22 (61.1%)	43 (71.7%)		9 (45%)	22 (73.3%)	
Education						
Illiterate	10 (27.8%)	10 (16.7%)	.35	6 (30%)	7 (23.3%)	.77
< Diploma	16 (44.4%)	27 (45%)		8 (40%)	15 (50%)	
≥ Diploma	10 (27.8%)	23 (38.3%)		6 (30%)	8 (26.7%)	
Pretransplantation Dialysis						
Yes	24 (66.7%)	44 (73.3%)	.48	14 (70%)	20 (66.7%)	.52
No	12 (33.3%)	16 (26.7%)		6 (30%)	10 (33.3%)	
Blood transfusion						
Yes	15 (41.7%)	22 (36.7%)	.62	8 (40%)	13 (43.3%)	.52
No	21 (58.3%)	38 (63.3%)		12 (60%)	17 (56.7%)	
Immunosuppressive regimen						
CAP	12 (33.3%)	18 (30%)	.45	8 (40%)	10 (33.3%)	.42
CMP	24 (66.7%)	42 (70%)		12 (60%)	20 (66.7%)	

Abbreviations: CAP, cyclosporine-azathiopine-prednisolone; CMP, cyclosporine–mycophenolate mofetil–prednisolone. *Statistically significant.

16.1% were seropositive for anti-CMV IgM and 11.4% were borderline.[14] The prevalence of CMV infection in our study was thus considerably higher than that in earlier reports. Another study by Sepehrvand et al demonstrated a 77.4% seroprevalence of anti-CMV IgG among hemodialysis patients, but only 7.1% were seropositive for anti-CMV IgM.[1] As expected, the prevalence of CMV infection was higher among renal transplant recipients than hemodialysis patients.

The high prevalence of CMV infection in transplant recipients may be due to changes in recipient T-cell subgroups. Cytomegalovirus usually exerts influences in hosts, suppressing the immune system, stimulating releasing of an interleukin inhibitor from monocytes, and rendering host cells susceptible to cytolysis by natural killer cells. Beyond the immunocompromised nature of transplant recipients, CMV-infected patients are at greater risk for opportunistic infections.[14]

Several studies have cited risk factors for primary CMV infection as blood transfusions, previously infected organs, and dialysis frequency in the week before transplantation.[1,13,15] We did not evaluate the frequency of dialysis before transplantation, but there was no significant relationship between pretransplant dialysis and CMV infection. Also, we failed to observe any correlation between blood transfusion and either CMV seropositivity or infection,

Table 2. Results of anti-CMV IgM and CMV DNA PCR in Transplant Recipients

	CMV DNA PCR Result	
	Positive	Negative
Anti-CMV IgM (ELISA method)		
Positive	19	17
Negative	1	13

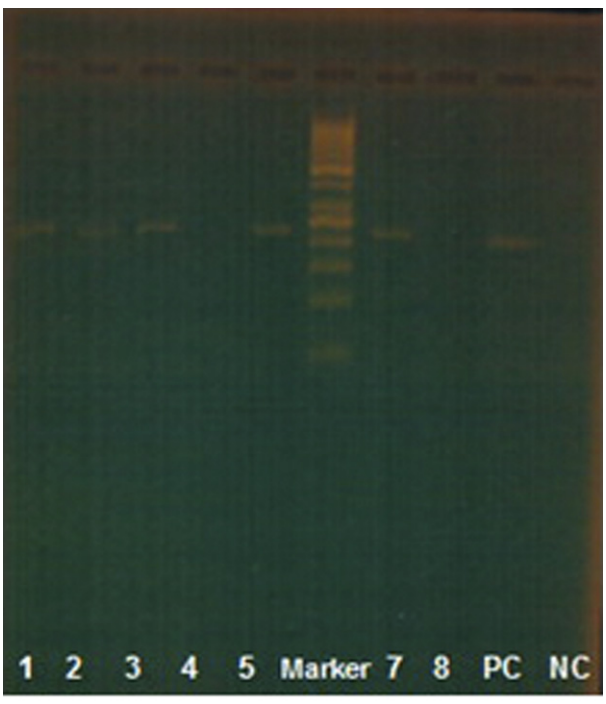

Fig 1. Amplification of PCR with electrophoresis by 2% agarose gel (a 222-base pair band was seen).

consistent with the findings of Akinbami et al in Lagos, Nigeria, in 2009.[16]

The donors and recipients were not investigated for probable CMV infection before transplantation, so we have no data regarding the probable route of transmission in CMV-infected cases. A prospective study could be helpful to investigate probable routes of CMV transmission in this population of patients.

In conclusion, the seroprevalence of CMV infection was high among renal transplant recipients of Urmia, Iran. The ELISA method offers appropriate sensitivity to screen recipients for CMV infection, but considering its relatively low specificity seropositive cases should be confirmed by PCR study.

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