

# Prevalence and Risk Factors of Recurrent Cytomegalovirus Infection in Kidney Transplant Recipients

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**Keywords.** cytomegalovirus infection, kidney transplantation, ganciclovir

**Introduction.** Recurrence of cytomegalovirus (CMV) infection following solid organ transplantation causes mortality and morbidity in allograft recipients. The aim of this study was to evaluate prevalence and risk factors of recurrent CMV infection in kidney transplant patients.

**Materials and Methods.** Four hundred and twenty-seven consecutive kidney transplant recipients were included in this retrospective cohort study. Both donors and recipients were CMV seropositive. Recurrent CMV infection (symptomatic or asymptomatic) was defined as detection of CMV infection in a patient who has had previously documented infection and who had not have virus detected for an interval of at least 4 weeks during active surveillance.

**Results.** Of 427 recipients, 71 (16.6%) had CMV infection, of which 19 (4.4%) were recurrent infection. Donor source, dialysis duration before transplantation, recipient and donor age and sex, and administration of antithymocyte globulin and prophylactic treatment ganciclovir were not associated with CMV infection or recurrence. The use of tacrolimus in the immunosuppressive regimen as compared to cyclosporine was an independent risk factor for CMV infection but not recurrent infection.

**Conclusions.** Intensive immunosuppressive regimen, such as using tacrolimus, might be associated with a higher risk for CMV infection, but this study was not able to document the same association for recurrent CMV disease. In patients receiving immunosuppressive regimens that include tacrolimus and antithymocyte globulin, prophylactic treatment for CMV disease with ganciclovir is recommended.

IJKD 2014;8:231-5  
www.ijkd.org

## INTRODUCTION

Cytomegalovirus (CMV) infection is one of the major infectious complications in kidney transplantation and is associated with acute rejection or chronic kidney allograft dysfunction and opportunistic infections.<sup>1</sup> Through its direct and indirect effects, CMV is associated with significant clinical illness, allograft loss, and mortality after

kidney transplantation.<sup>2-4</sup> Prophylaxis, treatment, diagnosis, and overall increasing awareness about CMV has improved over the past decade, and CMV disease is now generally diagnosed at an earlier stage than it was previously. Moreover, CMV infection is associated with an increased the risk of posttransplant lymphoproliferative diseases.<sup>5,6</sup>

Recurrent infection is defined as new detection

of CMV infection in a patient who had previously documented infection and who did not have virus detected for an interval of at least 4 weeks during active surveillance.<sup>7</sup> The strongest risk factor for CMV disease are CMV serostatus and lack of CMV-specific immunity. The combination of CMV seronegative recipients with CMV-seropositive donors leads to the highest risk of CMV disease.

The choice of antiviral therapy (preemptive or prophylactic, lasting for 3 or 6 months), as well as transplantation practices and immunosuppressive regimens, differs greatly between transplant centers. Patients can develop reactivation of this infection after transplantation in spite of previous exposure and the development of protective antibodies against CMV. Relapsing episodes of CMV infection occur in 23% to 33% of transplant patients, which is likely a reflection of incomplete suppression of viral replication following antiviral treatment with intravenous ganciclovir.<sup>8,9</sup> In this study, we investigated the prevalence and risk factors of recurrent CMV infection in kidney transplant patients.

## MATERIALS AND METHODS

### Participants

We retrospectively studied a consecutive of 427 kidney transplant recipients with stable kidney function (258 men and 169 women) from January 2010 to May 2012 at Shahid Labafiinejad Medical Center, Tehran, Iran. This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Written informed consent was obtained from all patients. All transplanted patients older than 18 years with documented CMV infection were enrolled in the study. The kidney donors were human leukocyte antigen antibody-mismatched (cadaver, related, and unrelated) donors. Immunosuppression for the recipients consisted of triple-drug therapy with tacrolimus (target level, 5 ng/mL to 7 ng/mL) or cyclosporine (target trough level, 100 ng/mL to 200 ng/mL), mycophenolate mofetil (500 mg thrice per day or 1 g twice daily), and prednisolone (5 mg/d). Prophylactic treatment with ganciclovir was initiated simultaneously with delivery of antithymocyte globulin. The ganciclovir dose was adjusted based on serum creatinine in cases of kidney function impairment.

The blood samples were obtained from recipients who were suspected to have CMV infection upon

clinical presentation, physical examination, and laboratory results. The disease was diagnosed according to the clinical features and detection of CMV antigen (pp65 antigenemia, more than 1/100 000 cells on immunofluorescent microscopy, IQ product kit, USA) in serum followed by quantitative CMV polymerase chain reaction viral load more than 2000 copy per milliliter (Qiagen kit).

The patients were treated with intravenous ganciclovir for 3 weeks providing dose reductions in patients with kidney function impairment. After treatment, the eradication was assumed by improvement of fever and clinical findings and it was confirmed by quantitative CMV polymerase chain reaction viral load until CMV-DNA was not detectable. Persistence of CMV-DNA after 21 days is associated with disease resistance.

Evidence of CMV infection with symptoms, classified as CMV syndrome, was recorded if any of the following was documented: fever higher than 38°C for 2 days or longer, new or increased malaise, leukopenia, 5% or more atypical lymphocytes, thrombocytopenia, hepatic transaminase elevation more than double the upper normal limit (non-liver transplant recipients only), and symptoms associated with CMV-positive blood culture, antigenemia, or DNA/RNA positivity, with no other identified cause of the symptoms and signs. A recording of tissue-invasive disease was made if the following were present: symptoms and signs of tissue invasion and CMV-positive biopsy specimen (eg, pneumonitis, hepatitis, retinitis, and gastrointestinal disease). Recurrent CMV infection was defined as CMV infection (symptomatic or asymptomatic) occurring after 3 months of transplantation after proven eradication of previous active CMV infection at the end of treatment (two negative samples separated by  $\geq 1$  week).<sup>6</sup>

### Statistical Analysis

The statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). The quantitative results were expressed as mean  $\pm$  standard deviation and were analyzed using independent Student *t* test. The statistical significance of differences was determined by the chi-square analysis with Yates correction for categorical variables. Significance was defined as a *P* value less than .05.

## RESULTS

Between October 2009 and 2011, 427 consecutive kidney allograft recipients were enrolled in this historical cohort study. The mean age of the recipients was  $39.8 \pm 14.9$  years (range, 16 to 77 years). Patients' characteristics are shown in Table 1.

The prevalence of CMV disease and recurrent CMV disease was 16% (71 of 427) and 4.4% (19 of 427), respectively. The recipients' age, sex, and duration of dialysis before transplantation were not significantly different between the two groups of patients with and without recurrent CMV infection. Cyclosporine serum level was in therapeutic level in all of the patients with CMV infection. History of immunosuppression before transplantation for the primary cause of kidney failure (lupus nephritis, glomerulonephritis, etc) was not correlated with the episode of CMV infection after transplantation. Immunosuppressive protocol

was detected to have an important role in CMV disease; recipients treated with tacrolimus were more likely to develop CMV disease compared with those on cyclosporine (odds ratio, 3.8; 95% confidence interval, 1.3 to 6.2;  $P = .01$ ), but it was not as an independent risk factor for recurrent CMV disease (26.3% versus 25%,  $P = .70$ ). There were 45 patients treated with tacrolimus. Antithymocyte globulin after transplantation did not show correlation neither with CMV disease nor with recurrent CMV disease (38% versus 28.5%,  $P = .07$  and 31.8% versus 38.5%,  $P = .09$ , respectively). Table 2 shows risk factors for CMV disease.

All of the patients were CMV-seropositive recipients from seropositive donors. Risk factors for recurrent CMV infection are shown in Table 3. Recipients' age, sex, and immunosuppressive regimen were not significantly different between groups.

**Table 1.** Characteristics of Kidney Transplant Recipients

Characteristic	Value*
Mean age, y	$39.2 \pm 15.7$
Sex	
Male	258 (60.4)
Female	169 (39.6)
Donor source	
Cadaver	107 (25.1)
Living unrelated	303 (71.0)
Living related	17 (4.0)
Pretransplant dialysis time, mo	
< 6	193 (45.2)
6 to 12	86 (20.1)
12 to 24	70 (16.4)
> 24	68 (15.9)
Cytomegalovirus disease	71 (16.6)
Recurrent cytomegalovirus disease	19 (4.4)

\*Values are frequencies (percentages), except for mean age.

**Table 2.** Risk Factors for Cytomegalovirus Infection\*

Parameter	Cytomegalovirus Infection		P
	Yes (n = 71)	No (n = 356)	
Recipient age, y	$39.4 \pm 15.1$	$38.0 \pm 16.4$	.70
Recipient sex			
Male	45 (63.4)	213 (60.0)	
Female	26 (36.6)	142 (40.0)	.60
Immunosuppression before transplant	6 (8.5)	41 (11.8)	.50
Antithymocyte globulin	27 (38.0)	101 (28.5)	.07
Immunosuppression regimen			
Cyclosporine	53 (74.6)	329 (92.4)	
Tacrolimus	18 (25.4)	27 (7.6)	.01

\*Values are frequencies (percentages), except for mean age.

**Table 3.** Risk Factors for Recurrent Cytomegalovirus Infection\*

Parameter	Recurrent Cytomegalovirus Infection		P
	Yes (n = 19)	No (n = 52)	
Recipient age, y	$38.3 \pm 16.8$	$39.9 \pm 14.3$	.70
Recipient sex			
Male	14 (73.7)	31 (59.6)	
Female	5 (26.3)	21 (40.4)	.10
Immunosuppression before transplant	2 (10)	5 (9.6)	.30
Antithymocyte globulin	7 (31.8)	20 (38.5)	.90
Immunosuppression regimen			
Cyclosporine	14 (73.7)	39 (75)	
Tacrolimus	5 (26.3)	13 (25)	.70

\*Values are frequencies (percentages), except for mean age.

## DISCUSSION

Cytomegalovirus infection is one of the most common infections following kidney transplantation. It is considered as a potential contributor to graft loss and a cause of severe mortality and morbidity.<sup>8</sup> Without intervention and preventative therapy, symptomatic CMV infection can develop in 20% to 60% of kidney transplant recipients, of which the majority of CMV replication and disease is reported early during the first 3 months after transplantation at the time of the highest immunosuppressive load.<sup>8</sup> In the present study, the prevalence of CMV disease and recurrent CMV disease was 16% and 4.4%, respectively. These rates were lower than that reported by Bouedjoro-Camus and colleagues; the prevalence of CMV disease was 26.5% in sera of their 192 kidney allograft recipients.<sup>10</sup> Watcharanan and colleagues showed symptomatic CMV infection in 18 patients (4.6%).<sup>11</sup>

CMV seronegative recipients receiving solid organ transplants from CMV-infected seropositive donors are at the highest risk for CMV replication and disease. The risk also increases in CMV seropositive recipients treated with T-cell- or B-cell-depleting antibody regimens administered for induction or rejection. Previous studies demonstrated CMV infection was more frequent in allograft recipients receiving transplants from seropositive donors, but our patients were all CMV-seropositive recipients from seropositive donors, a second risk factor for CMV infection, which is highly prevalent in Iran.<sup>12</sup> Sawyer and coworkers evaluated 535 kidney transplant patients and found that recurrent tissue-invasive disease was higher with cadaveric kidney transplant than living donor kidney transplant.<sup>13</sup> Helanterä and colleagues studied on 254 kidney transplant patients and demonstrated that the level of immunosuppression did not predict recurrence of CMV infection.<sup>14</sup>

It is now accepted that high-risk patients benefit from prophylaxis regimens, particularly ganciclovir. The most appropriate CMV-therapy following kidney transplantation remains debatable. We have previously reported that CMV prophylaxis with oral ganciclovir for 12 weeks has the same outcome as intravenous ganciclovir with no serious side effects. Both regimens relatively reduce the rate of CMV infections.<sup>12</sup> Some studies concluded that both prophylactic and preemptive approaches were effective to manage CMV for the first year

posttransplant. In a classic paper, Lowance and coworkers reported that high-dose valaciclovir prophylaxis reduced not only CMV disease, but also the number of acute rejection episodes in CMV-seronegative kidney transplant recipients from seropositive donors.<sup>15</sup>

The results of our study demonstrate that high immunosuppressive regimen is associated with a higher risk for CMV infection. Recipients treated with tacrolimus were more likely to develop CMV disease compared with cyclosporine because patients treated with tacrolimus did not receive ganciclovir prophylaxis for CMV infection, but after CMV treatment recurrence was not more frequent in the patients. Antithymocyte globulin after transplantation did not show correlation with CMV disease or recurrent CMV disease. This might be because the sample size was not enough. In patients receiving higher immunosuppressive regimens such as tacrolimus and antithymocyte globulin, prophylactic treatment with ganciclovir is recommended for CMV disease.<sup>16,17</sup>

Severely immunosuppressed transplant recipients may show faster CMV dynamics, delayed clearance, and more recurrence episodes.<sup>18</sup> It is shown that rates of CMV-resistant genotypes are higher in highly immunosuppressed patients such as bone marrow recipients.<sup>19</sup> It is believed that the first episode of CMV replication should be treated with sufficient dosing of antivirals, without modifying maintenance immunosuppression. In cases of recurrence, antiviral treatment activating stimuli should be controlled and combined with moderately reduced immunosuppression since CMV-specific immunity might be inadequate.<sup>20</sup>

Our study had some limitations: first, the sample size might not be enough to show the impact of antithymocyte globulin on CMV recurrence. Second, due to retrospective design of the study, we could not evaluate the pretreatment CMV viral load among the study groups. Our result should be confirmed with a larger prospective study.

## CONCLUSIONS

Along with immunosuppression protocols, prophylactic and therapeutic management of CMV infection is necessary for kidney transplant recipients, especially in highly immunosuppressed patients. Our study showed that intensive immunosuppressive regimen was associated with

a higher risk for CMV infection. Recipients treated with tacrolimus were more likely to develop CMV disease as compared with cyclosporine, but it was not as an independent risk factor for recurrent CMV disease after treatment. In patients receiving higher immunosuppressive regimens such as tacrolimus and antithymocyte globulin, prophylactic treatment with ganciclovir is recommended.

### CONFLICT OF INTEREST

None declared.

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Received May 2013

Revised October 2013

Accepted November 2013