CYTOMEGALOVIRUS DISEASE IN RENAL ALLOGRAFT RECIPIENTS: EXPERIENCE OF A SINGLE CENTER

Tung-Wei Hung*, Horng-Rong Chang**, Sheng-Wen Wu*, Chih-Kuang Lin*, Jong-Da Lian*

Background: This analysis is to investigate the clinical presentations of cytomegalovirus (CMV) disease in kidney transplant recipients.

Methods: We conducted the CMV polymerase chain reaction (PCR) survey among kidney transplant recipients if the patient presented with suspected symptoms, including fever, hematologic abnormalities, gastrointestinal symptoms, abnormal liver function, and allograft dysfunction from January 2002 to December 2004. Direct CMV detection was performed in urine and blood samples by a qualitative PCR method. CMV disease was defined as CMV-associated symptoms with positive CMV PCR. The clinical presentations and outcomes were reviewed.

Results: Of the 164 kidney transplant recipients, we detected 10 patients (6.1%) with CMV disease. The CMV disease was diagnosed at 2.4 ± 1.3 months post transplantation. The clinical symptoms and signs included fever in 10 patients, elevated serum creatinine in 6, raised liver enzyme in 5, abdominal pain in 4, vomiting in 2, diarrhea in 2, thrombocytopenia in 4, leucopenia in 4, and ischemic colitis with mortality in 1. All the patients received intravenous gancyclovir with clinical response except one died from ischemic colitis.

Conclusion: The possibility of CMV disease should be explored if a kidney transplant patient presented with fever and/or CMV associated symptoms within 6 months post transplantation. It is beneficial if CMV disease can be diagnosed early so that adequate antiviral therapy can be offered. (Acta Nephrologica 2009; 23: 21-25)

INTRODUCTION

Although renal transplantation has been much more successful thanks to the development of newer immunosuppressants, post-transplant opportunistic infections remain an important issue. Immune suppression renders the transplant recipient susceptible to a broad array of opportunistic infection. CMV is a member of the genus Herpesvirus and belongs to the family Herpesviridae and is one of the most important infections in renal transplant recipients.

CMV infection has been defined as either tissue or antigen detection of CMV in the patient, whereas CMV disease has been defined as symptomatic organ system or systemic involvement resulting from CMV infection. The diagnosis of “CMV disease” requires clinical signs and symptoms, such as fever, leukopenia, or organ involvement including hepatitis, pneumonitis, pancreatitis, colitis, meningoencephalitis, and rarely myocarditis. Encephalopathy and chorioretinitis are unusual in renal transplant recipients. The infection is notorious and involves major organs including the liver, lung, and gastrointestinal (GI) tract. It can cause clinical disorders from allograft dysfunction to systemic involvement. CMV infection presents most often as asymptomatic viremia or with fever and neutropenia. The clinical manifestations of CMV disease are often nonspecific. Consequently, careful interpretation of presenting signs and symptoms with appropriate microbiologic data are important in this patient group.

The presence of CMV IgM, 4-fold increase of CMV IgG, and CMV antigen are conventional indicators of CMV infection. There is a high prevalence of past infection before transplantation and the serologic response can be dramatically influenced by the immunosuppressed state. Transplant recipients may fail to produce an antibody response despite viremia. Serological method is less sensitive due to delayed seroconversion and less specific. The protracted time allow the undetected and untreated disease to progress. CMV assays...
that are sufficiently sensitive and specific are required for optimal surveillance and management of CMV infection. In the last decade, microbiologic assays with CMV antigen (pp65) or polymerase chain reaction (PCR) for detecting CMV DNA, which are indicative of active viral replication, are employed for diagnosing CMV infection, in contrast to serologic testing and viral cultures from multiple sites used previously. In the study of Jigna Bhatia et al.,

the sensitivity and specificity of serologic test (CMV IgM) were 72.97 and 62.06%; those of antigemia assay were 89.18 and 100% and those of PCR were 100 and 72.41%, respectively. CMV-PCR uses DNA that neither deteriorates nor diminished with storage. The pp65 antigemia assay deteriorates rapidly – even with a 6-hour delay of processing the sample. Early CMV PCR detection followed by correct antiviral therapy can prevent or treat CMV disease. Qualitative CMV PCR is the test for CMV infection employed at our institution.

This analysis on CMV disease in kidney transplant recipients is the experience of only a single center.

PATIENTS AND METHODS

From January 2002 to December 2004, 164 patients underwent kidney transplant surgery. All of the patients received standard course of antilymphocyte antibody treatment and kept receiving triple-drug therapy after transplantation with prednisolone, mycophenolate mofetil/mycophenolic acid, and cyclosporine/tacrolimus prescribed. No prophylactic agents were used for CMV. If the patient presented with CMV-related symptoms, such as (1) fever without an obvious cause for 3 days, (2) GI tract involvement; (3) pulmonary complaints; (4) hematologic involvement with either leucopenia (WBC < 4000/mm³), thrombocytopenia (platelets < 100,000/mm³); 5) a 25% rise in serum creatinine (Scr.; (6) liver function derangement, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 40 IU/L. We conducted the CMV PCR survey in urine and blood sample. If the patients presented with GI symptoms, the upper or lower GI endoscopic examination with biopsy was performed.

Details of CMV PCR have been reported previously. In brief, each PCR reaction mix (50 μl) contained 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.001% gelatin, 200 μmol/L each of dATP, dCTP, and dGTP, 400 μmol/L dUTP, 20 pmol of each oligonucleotide primer, 20 molecules of the HHVQ-1 ICS standard, 2.5 units of Taq polymerase (PE Applied Biosystems, Foster City, CA), and DNA from 10 μl of whole blood. Separate reaction vessels were prepared with primers specific for CMV. A master mix containing enough reagents for nine reactions without the oligonucleotide primers was prepared. It was then aliquoted into each of the eight PCR reaction tubes containing the virus-specific primers. Complete reactions were proceeded in a GeneAmp PCR System 9600 thermocycler (PE Applied Biosystems) and amplified under the following conditions: 1 cycle of 2 minutes at 95°C, 36 cycles of 30 seconds at 94°C, 30 seconds at 65°C, and 1 minute at 72°C, followed by a final extension of 9 minutes at 72°C. After amplification, PCR products were separated by agarose gel electrophoresis, identified by staining with SYBR Gold (Molecular Probes, Eugene, OR). The sensitivity is 100% and the specificity is 90%.

In our series, CMV disease was defined as positive laboratory test results (qualitative CMV PCR) with symptoms of organ involvement such as fever, hematologic abnormalities, gastrointestinal symptoms, abnormal liver function, allograft dysfunction or a clear-cut histologic evidence on biopsy of involved organ (e.g. GI tract).

Patients diagnosed with CMV disease were enrolled in this analysis. Intravenous ganciclovir at a dosage of 5 mg/kg twice daily adjusted to the glomerular filtration rate was administered for the first 2 weeks followed by 2-month course of oral ganciclovir. Clinical data were retrieved by reviewing medical records of the patients.

RESULTS

Of the 164 kidney transplant recipients, 10 (6.1%) were diagnosed as having CMV disease. On average, the diagnosis was made at 2.4 ± 1.3 months post transplantation and the CMV disease resolved after 2-week intravenous ganciclovir and reduction in immunosuppression. There were different clinical presentations including subclinical esophageal ulcer with dysphagia, symptomatic abdominal pain, to fatal ischemic colitis. The following symptoms and signs were present during hospitalization for CMV disease (Table 1): fever for 11.1 ± 10.7 days (range: 3-31) in 10 patients, abnormal liver function in 5 with ALT 229 ± 307.8 (range: 13-800) U/L, leucopenia (WBC < 4,000/mm³) in 4, thrombocytopenia (platelet < 100,000/mm³) in 4, GI involvement in 7, raised SCR in 6, and 1 died of CMV disease-related ischemic colitis. In the nine symptomatic survivors, the baseline of serum Cr. is 1.3 ± 0.4 mg/dl. It rose to 2.7 ± 1.7 mg/dl (p = 0.031 vs. baseline) during the course of CMV disease, but returned to 1.4 ± 1.5 mg/dl (p = 0.394 vs. baseline) at the 4.2 ± 3.8 months after CMV infection. Among them one diabetic renal transplant recipient experienced an episode of acute allograft rejection in the 6th month post-transplant when there was an attempt at steroid with-
Table 1. Clinical presentations of CMV disease in this analysis

<table>
<thead>
<tr>
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<th>Percent(n) in this study</th>
<th>Percent*</th>
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<tr>
<td>CMV disease</td>
<td>6.1 (10)</td>
<td>3.613, 8.519, 17.632, 33.33, 35.30, 20-6014,31</td>
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<tr>
<td>Posttransplant duration (months)</td>
<td>2.4±1.3</td>
<td>1-410, 2-619, 2.416, 3.446, 3.530, 5.244</td>
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<td>Fever</td>
<td>100 (10)</td>
<td>4313, 7035, 7136, 7619, 9118, 10020</td>
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<tr>
<td>Leucopenia (WBC &lt; 4,000/mm)</td>
<td>40 (4)</td>
<td>2113, 5219, 8218, 83.330,</td>
</tr>
<tr>
<td>Thrombocytopenia (platelet &lt; 10^9/mm)</td>
<td>40 (4)</td>
<td>2719, 4731, 5219</td>
</tr>
<tr>
<td>Abnormal liver function</td>
<td>50 (5)</td>
<td>2113, 4819, 70.320</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>70 (7)</td>
<td>1016, 1319, 2919, 10-3018</td>
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<tr>
<td>Allograft dysfunction</td>
<td>60 (6)</td>
<td>18.5530, 5231</td>
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<tr>
<td>Pneumonitis</td>
<td>0 (0)</td>
<td>914, 1019, 1749, 22.220</td>
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<td>Death</td>
<td>10 (1)</td>
<td>21930, 18.833</td>
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a: reference number

Among the seven patients with GI involvement, endoscopic examination and biopsy were performed with histologic identification of CMV inclusion bodies. We reported a diabetic renal transplant recipient with CMV colonic ulcerations who presented with tarry stool diarrhea. Another 28-year-old male presented with intermittent fever, nausea, and vomiting since the 54th day post-transplant. Upper gastroenteroscopy showed acute gastric, duodenal ulcers, and numerous gastric polyps causing pyloric obstruction. One patient with fatal ischemic colitis had fever of unknown origin for 27 days followed by progressive right lower abdominal pain. The clinical condition deteriorated rapidly with the development of disseminated intravascular coagulopathy and internal bleeding despite right hemicolectomy and antiviral therapy. The patient died 11 days after the onset of abdominal pain.

These patients with CMV disease were treated with intravenous ganciclovir for 2 weeks. The graft and patient survival was 90% at the 7.8 ± 3.3 months post-transplantation, which is consistent with previous reports.

Symptomatic CMV infection occurs in 20-60% of all transplant recipients and is a significant cause of increased morbidity and mortality in this population. We report a 6.1% frequency of CMV disease at our center from January 2002 to December 2004. Specific immunosuppressive agents including antithymocyte globulin and mycophenolate mofetil have been suggested as increasing risk for CMV infection. The onset of disease usually follows the period of maximal immunosuppression. The incidence may be somewhat lower in kidney transplant recipients who do not receive antilymphocyte therapy, high-dose mycophenolate mofetil, or the potent combination of mycophenolate mofetil and tacrolimus with adequate antiviral prophylaxis.

CMV disease presents most commonly as a febrile illness, but can present with only blood abnormalities (leukopenia, thrombocytopenia, anemia, and elevated liver enzymes) or with symptoms of solid organ involvement (pneumonia, retinitis, and colitis). Durlik et al reported that the most frequent clinical manifestations of CMV disease were fever (91%), leukopenia (82%), and thrombocytopenia (27%). The manifestations of CMV disease in our series included fever (100%), GI involvement (70%), elevated creatinine (60%) and liver enzyme (50%), leukopenia (40%) and thrombocytopenia (40%). There was no pneumonia in our series, which is also agreement with other studies.

GI involvement was a significant finding in our series. CMV disease with GI involvement is present in 2-6% of renal transplant recipients. Different areas of the entire GI tract, from the oropharynx to the anus, may be involved. The symptoms and signs vary with the affected gut segment and may include dysphagia, odyno-
phagia, nausea, vomiting, abdominal pain, GI bleeding, perforation, or diarrhea. Ulcerations, erosions, and mucosal hemorrhage are the primary macroscopic findings. Typical endoscopic findings of tissue-invasive CMV of the digestive tract are shallow, erythematous erosions or localized ulcers, and less commonly, plaques, nodules, and polyps. However, these visual findings are not specific, so biopsy is essential. Pathologic examination of the involved gut typically reveals diffuse ulcerations and necrosis with scattered CMV inclusions which play an active role in damaging the colonic mucosa, primarily as a result of CMV vasculitis. The virus can proliferate in vascular endothelial cells, leading to vasculitis and small vessel thrombosis with local ulceration. Gastrointestinal bleeding among renal transplant recipients is commonly caused by erosions due to CMV. In our patients, CMV inclusion bodies on the mucosal biopsy specimen were discovered through endoscopic or colonscopic examination. We had a rare case of CMV disease with GI involvement presented with pyloric obstruction in the early posttransplant months and another diabetic renal transplant recipient with CMV disease presented with tarry diarrhea because of multiple colonic ulcerations. Early endoscopicscopic examination is needed for transplant recipients with chronic diarrhea associated with anemia and that biopsy is necessary for diagnosing CMV disease.

According to our analysis, patients who experience CMV disease have poorer renal function at the time of CMV diagnosis. Whether the virus itself can cause allograft dysfunction is controversial. Renal function may deteriorate in patients with CMV infection but factors such as decreased renal perfusion, acute tubular necrosis, and transplant rejection may be more important than a direct viral effect on the kidney. Several studies have reported that CMV infection and disease are associated with clinical acute rejection in kidney allograft recipients. In addition, CMV disease also seems to play a role in the pathogenesis of chronic rejection, possibly due to the inability to adequately treat acute rejection or to the increased virulence of a latent CMV virus in recipients being treated for acute rejection. We reported a diabetic renal transplant recipient who experienced an episode of acute allograft rejection in the 6th month posttransplant when there was an attempt at steroid withdrawal for the CMV disease with GI involvement. The acute rejection was steroid resistant. CMV disease may be associated with acute or chronic allograft rejection, thus tapering of the immunosuppression needs to be done cautiously.

In our series, one patient died from CMV ischemic colitis with presentation of fever of unknown origin in the early months post transplantation. If the diagnosis and treatment of CMV disease can be made earlier, it may be lifesaving. Fever in immunocompromised transplant recipients within the first few months of surgery should be considered an emergency because it is the principal manifestation of serious infection. Symptoms of systemic CMV infection in immunocompromised patients are similar to classical acute infection. In our experience, the CMV-PCR detection should be performed to rule out CMV infection if the patients present with persistent fever during broad-spectrum antimicrobial therapy. The CMV-PCR is a technique for early and rapid diagnosis of CMV infection.

Current antiviral management is highly effective in managing CMV infection following kidney transplantation. Management consists of preventive (prophylactic and/or pre-emptive therapy) and therapeutic treatment of established disease. Avoiding CMV sero-mismatching, ie., D+/R- and blood products from CMV seropositive donor may reduce the risk of CMV infection. According to the Canadian consensus guidelines, transplant patients from donors positive (D+) for CMV into recipients negative (R-) for CMV and CMV-seropositive recipients treated with ATG or OKT3 for induction or treatment of acute rejection should receive antiviral prophylaxis therapy. Pre-emptive antiviral therapy is attractive in that it can minimize both toxicity and costs. The therapy involves periodic PCR monitoring for CMV DNAemia or CMV pp65 antigenemia monitoring of whole blood. This is associated with early treatment and reduction in severity and mortality of disease.

Patients with symptomatic CMV infection should receive intravenous ganciclovir, which should be continued until clearance of viremia monitored by pp65 antigenemia or quantitative PCR. The usual course comprises 2-4 weeks of intravenous therapy. Fishman and Rubin advocated the use of intravenous ganciclovir followed by a 2-month course of oral ganciclovir in seropositive individuals and for 3-4 months in those with primary infection. Alternative therapies (not Food and Drug Administration approved for use in solid organ transplant recipients) include foscarner, cidofovir, and leflunomide, which are reserved for treatment of antiviral resistance.

In summary, we suggest that the possibility of CMV disease should be explored if a kidney transplant recipient presents with fever of unknown origin and/or CMV associated symptoms within 6 months post transplantation. It is beneficial if CMV disease is diagnosed early so that adequate antiviral therapy can be given.

REFERENCES