Clinical Utility of Viral Load in the Management of Cytomegalovirus Infection in Solid Organ Transplant Patients in Kuwait

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ABSTRACT

Background. Infection with human cytomegalovirus (CMV) in solid organ transplant patients remains an unresolved challenge, despite improvements in immunosuppressive therapy, post-transplantation care, viral prevention, and therapy.

Methods. We conducted quantitative real-time polymerase chain reaction (PCR) assays of CMV on plasma samples of 1,168 patients in Kuwait who received solid organ transplants from 2012 to 2014 to detect and monitor CMV DNA viral load.

Results. Of the 1,168 patients, 180 (15.4%) were positive for CMV DNA. Among the CMV DNA–positive patients, 119 (66.1%) remained without symptoms and 61 (33.9%) developed CMV-related symptoms. During the follow-up period, peak viral loads were significantly ($P < .05$) higher in symptomatic patients (mean 970 copies/mL; range, 15–625,000 copies/mL) than in asymptomatic patients (<150 copies/mL; range, 67–2,650 copies/mL). Many symptomatic patients ($n = 57$) were successfully treated, and their viral loads declined. However, some symptomatic patients had irregular viral-load kinetics, with prolonged periods of symptoms despite CMV treatment; we excluded the possibility of drug resistance in these patients, because there was no evidence of clinical resistance to treatment.

Conclusions. Quantitative real-time PCR of CMV DNA is useful in monitoring CMV infection and the effectiveness of CMV treatment in renal transplant recipients in Kuwait.

CYTOMEGALOVIRUS (CMV) is one of the commonest pathogens that infect solid organ transplant (SOT) patients [1]. CMV infection in SOT patients can cause prolonged fever, leukopenia, hepatitis, colitis, retinitis, and allograft injury, resulting in significant morbidity and, occasionally, mortality [2,3]. Owing to the magnitude of the direct and indirect effects of CMV infection, considerable effort has been aimed at defining strategies for its prevention and treatment [1]. Compared with the antigenemia assay, quantitative nucleic acid testing for CMV is the main option for diagnosis to decide whether to initiate preemptive therapy and to monitor the response to therapy [4]. Viral load testing by means of real-time polymerase chain reaction (PCR) assay has been shown to improve rapid diagnosis and monitoring of clinical responses to antiviral therapy [5–7]. This assay has better precision, broader linear range, faster turnaround time, higher throughput, and less risk of carryover contamination than does conventional PCR [8].

As a follow-up to our previous study establishing sensitive and specific real-time PCR for quantitation of CMV DNA in clinical specimens [9], the present retrospective study was aimed at demonstrating the clinical utility of viral load monitoring of CMV by means of real-time PCR in SOT patients in Kuwait.

MATERIALS AND METHODS

Study Population

A total of 1,168 patients (742 male and 426 female; median age, 48 years) who received SOTs in the years 2012–2014 at the Organ Transplant Center, Ministry of Health, Kuwait, were enrolled in this study. All patients underwent routine monitoring of

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Table 1. Demographic Characteristics of Single Organ Transplant Patients (n = 1,168)

Characteristic	n (%)
Age (y)	
Mean	45
Median	48
Sex	
Male	742 (63.5)
Female	426 (36.5)
Nationality	
Kuwaiti	879 (75.3)
Non-Kuwaiti	289 (24.7)
Solid organ transplant	
Kidney	1,165 (99.7)
Lung	2 (0.17)
Kidney + pancreas	1 (0.09)
CMV DNA negative	988 (84.6%)
CMV DNA positive	180 (15.4%)
Asymptomatic	119/180 (66.1%)
Symptomatic	61/180 (33.9%)
Graft rejection	25/61 (41%)
Systemic CMV disease	21/61 (34.4%)
Leukopenia	7/61 (11.5%)
Fever	6/61 (9.8%)
Fever + leukopenia	2/61 (3.3%)
Death	1/61 (1.6%)

Abbreviation: CMV, cytomegalovirus.

post-transplantation CMV viral load. Serial blood samples were collected every week until 14 months after transplantation and tested for CMV infection and viral load by means of quantitative real-time PCR assays. Data were collected regarding demographic factors, antiviral treatment, and occurrence of CMV disease. All patients received the following immunosuppressive treatment, depending on their condition, to reduce the risk of organ rejection: (1) induction therapy with interleukin-2 receptor blockers (Simulect [basiliximab] and Zenapax [daclizumab]); antithymocyte globulin was administered to high-risk patients only; (2) maintenance therapy with prednisolone, Neoral (cyclosporine), Cellcept (mycophenolate mofetil), and Prograft (tacrolimus). The patients received antiviral drugs according to the antiviral chemotherapy protocol followed in the Organ Transplant Center: (1) prophylactic therapy with ganciclovir (GCV) intravenously (IV; 5 mg/kg twice daily) or oral valganciclovir (VGCV; 900 mg/d) for 2 weeks; (2) preemptive therapy with GCV IV (5 mg/kg twice daily) or VGCV (900 mg/d) for 2 weeks; (3) direct therapy with GCV IV (5 mg/kg twice daily) or VGCV (900 mg twice daily) for 3 weeks followed by either oral GCV (1 g 3×/d), or VGCV (900 mg/d) until clinical resolution; and (4) maintenance with oral GCV (1 g 3×/d) and then oral VGCV (900 mg/d) for 3 months to 1 year.

DNA Extraction

Plasma was separated from whole blood, and DNA was extracted from plasma samples with the use of Roche Magna Pure LC system (Roche Diagnostics, Indianapolis, Indiana) according to the manufacturer's instructions. To control viral DNA isolation and detection efficiency, 10 µL Light Cycler CMV Internal Control DNA was added to 200 µL of sample and carried though the DNA extraction step preceding PCR amplification.

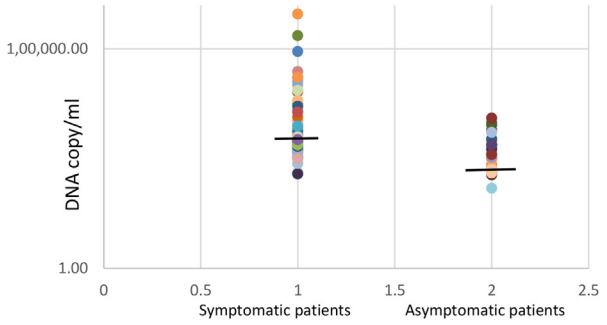

Fig 1. Median value of peak viral load of symptomatic and asymptomatic cytomegalovirus infections as demonstrated by means of real-time polymerase chain reaction.

CMV Quantitative Real-Time PCR Assays

Quantitation of CMV viral load by means of real-time PCR assays were performed in the Virology Unit, Microbiology Department, Kuwait University, Kuwait. The procedure, in brief, was as follows. The isolated CMV DNA was eluted in a volume of 100 µL. For viral DNA amplification and quantitation, 2 real-time systems were used: Light Cycler 2.0 (Roche Diagnostics, Mannheim, Germany) and Cobas Ampliprep/Cobas Taqman (Roche Diagnostics). For the Light Cycler 2.0 system, 10 µL eluted viral DNA was mixed with 15 µL master mix from the Artus CMV LC PCR Kit (Qiagen, Hilden, Germany). An internal control was included to control for DNA isolation efficiency and to check for possible PCR inhibition. CMV DNA quantitation was performed according to the manufacturer's instruction. The lower limit of detection of the assay was <650 copies/mL. For Cobas Ampliprep/Cobas Taqman system, 550 µL of plasma sample was used for the assay, and CMV DNA quantification was performed according to the manufacturer's instructions. The lower limit of detection of that assay was <150 copies/mL.

RESULTS

Of the 1,168 SOT patients involved in this study, 742 (63.5%) were male and 426 (36.5%) female, with an overall average age of 45 years. Among these patients, 879 (75.3%) were Kuwaiti and 289 (24.7%) non-Kuwaiti (Table 1). The total number of samples taken from all patients during follow-up was 3,098. Kidney transplantation was the most common procedure (n = 1,165; 99.7%); other transplantations included 2 lung (0.17%), and 1 kidney plus pancreas (0.09%). The donor/recipient CMV serostatus of the 1,168 SOT patients involved in this study were as follows: D+/R+ (n = 1157; 99%), D+/R− (n = 11; 1%). One hundred eighty SOT patients (15.4%) were positive for CMV DNA according to real-time PCR assays after a period of up to 14 months after transplantation, with a median follow-up period of 120 days; however, 988 patients (84.6%) remained negative. Eight hundred sixty-six samples were collected from the 180 CMV-infected patients, with an average of 5 samples per patient. Among the CMV DNA–positive patients, 119 (66.1%) remained without symptoms and 61 (33.9%) developed CMV-related