

The first two ganciclovir resistant cytomegalovirus isolates from kidney and pediatric stem cell transplant recipients in Turkey

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Abstract

Background: The emergence of cytomegalovirus (CMV) resistance to antivirals causes an increasing problem in transplant recipients, complicating therapeutic and clinical management. Widely used antivirals for therapy, preemptive therapy, and prophylaxis are the causes for resistance. This study aimed to report the first two transplant recipients who had CMV ganciclovir (GCV) resistance assessed by UL97 viral gene sequencing in Turkey.

Case presentation: CMV infection was monitored by plasma quantitative viral DNA real-time polymerase chain reaction (Abbott Molecular Inc., Illinois, USA) in the transplant recipients. Sequence analysis of CMV UL97 in CMV DNA-positive cases for GCV resistance was conducted, and while looking for UL97 GCV resistance in Ege University hospital, the first renal transplantation recipient with CMV mutation in Turkey was detected. Also, a stem cell transplant recipient was the first case for pediatric stem cell transplantation having UL97 GCV-resistant strain in Turkey. A594V and C603W mutations were the detected mutations, respectively.

Conclusion: Using GCV/valganciclovir (VGCV) for preemptive therapy, prophylaxis, treatment, and especially maintenance therapy for a long time with VGCV when CMV DNA is still detectable in peripheral blood might represent a risk factor for the emergence of CMV GCV resistance. The present findings indicate that resistance to widely used GCV as therapy, preemptive therapy, and prophylaxis should be monitored carefully in transplant recipients routinely for good patient management and effective antiviral therapy.

Key words: Cytomegalovirus, ganciclovir resistance, mutation, UL97

Introduction

Human cytomegalovirus (CMV) is a serious complication for the immune-suppressed transplant recipients. Ganciclovir (GCV), used for treating CMV infection, is a deoxyguanosine analog and the first drug of choice in treating CMV infection in immunosuppressive patients. Recently, due to the widespread use of antiviral therapy, problems related to

CMV drug resistance are most likely to develop especially in transplant recipients (1-5). In solid organ transplant recipients, although drug resistance varies according to the organ transplanted and immunosuppressive treatment, it is usually between 5% and 10% in CMV seronegative recipients treated for CMV infection and higher in lung transplant recipients (4-8).

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Because GCV is the first drug of choice in prophylaxis and preemptive therapy, UL97 resistance mutations are the most frequent mutations. With the development of resistance, antiviral therapy becomes completely ineffective, and it may produce results according to the condition of immunosuppression of the host, ranging from asymptomatic infection to the fatal disease.

In the case of clinical suspicion of drug resistance, it is recommended to confirm it with a laboratory test. The need for rapid diagnosis of drug resistance has led to the development of genotypic methods for determining the resistance mutations (9).

This study aimed to report the first two transplant recipients who had CMV GCV resistance assessed by UL97 viral gene sequencing in Turkey: a renal transplantation recipient and a pediatric stem cell recipient with CMV mutation.

Material and methods

Quantitative CMV DNA was detected with a Fluorion CMV QNP 3.0 Real-Time PCR Kit (iontek -Mag 16, iontek A.Ş, İstanbul) in samples in which nucleic acid isolation was performed. Then, the mutations defined for GCV (M460V, M460I, H520Q, A591V, C592G, A594V, A594T, L595S, E596G, K599T, C603V, C607F, and C607Y) were investigated with the help of UL97 sequence analysis. For the evaluation of UL97 gene region, ABI 3130 was used. The primers CMV_UL97_F GTTTCCACACAGACATGTTT and CMV_UL97_R GCATTCGTGGTAGAAGC were used in the analysis of a sequence of 700 base pairs. The genetic comparison was done with CMV AD169 strain.

CASE 1

A 17-year-old renal transplant recipient had a CMV infection posttransplant on day 35. Because he had a rise in serum creatinine levels, he received GCV [10 mg/(kg - day)] for 8 days, and valganciclovir (VGCV) (900 mg/day) was used afterward. During the follow-up when the patient was using VGCV, CMV DNA became positive (11,790 IU/mL) on day 175. After receiving GCV therapy for 20 days, VGCV was started again using the same doses. Because CMV DNA was still positive (2,138,500 IU/mL) on day 193, UL97 sequencing was done for GCV resistance and CMV UL97 A594V mutation was found. The same mutation was found in

plasma samples collected on days 175 and 182 when analyzed retrospectively. In kidney biopsy specimens, microangiopathic and tubular changes were seen in the graft tissue. The basal values of creatinine were stable, and therefore immunosuppressive medication doses were decreased. The patient was followed up with CMV DNA measurements, and CMV DNA values decreased in time.

CASE 2

Stem cell transplantation was performed for Fanconi aplastic anemia in an 11-year-old girl. The recipient was seropositive, but the donor was seronegative before transplantation. The leukocyte engraftment date was 18 days, and the thrombocyte engraftment date was 31 days posttransplantation. Chimerism was 100%. CMV DNA was found (1587 IU/mL) on posttransplantation day 10. Oral VGCV was started at a dose of 30 mg/(kg - day). One month posttransplantation, the patient suffered from graft versus host disease. In the second month, VGCV therapy was stopped when CMV DNA was found negative in two consecutive plasma samples. After 45 days, CMV DNA levels rose to 2086 IU/mL, and GCV therapy was started at a dose of 10 mg/(kg - day). CMV DNA levels were always positive (<1000 IU/mL) till the 10th month posttransplantation even after maintenance therapy with VGCV. When levels rose to 2,197,584 IU/mL in the 10th month posttransplantation, CMV C603W mutation was observed (posttransplantation day 319), and foscarnet [180 mg/(kg - day)] for 2 weeks followed by 90 mg/(kg - day) with CMV HIG [(100 mg/(kg - week)] was started. CMV DNA levels dropped to 2578 IU/mL in 2 weeks after therapy. Unfortunately the patient died because of bronchiolitis obliterans, pneumonia, and pulmonary bleeding on day 380 posttransplantation.

Results and Discussion

The clinical, therapeutic, and virological characteristics of these patients are listed in Table 1. The renal transplant patient had posttransplant CMV infection. He received GCV and VGCV therapy. Since CMV DNA levels were high during therapy, sequence analysis was done for GCV resistance and A594V mutation was found. The patient was the first renal transplant case with GCV resistance in Turkey. It was reported that A594V was the most frequent mutation (10, 11). Seropositive

Table 1. Clinical, therapeutic, and virological characteristics of the patients.

P	Sex/Age (year)	Tx	CMV D/R	CMV inf (days PTx)	Clinical condition	Antiviral	Dose [(mg/(kg · day)]	GCV R (days Pt)	Outcome
1	M/17	Renal	+/+	35	Creatinine levels ↑↑	GCV→ Oral VGCV	GCV 10 Oral VGCV*	193 Mutation: A594V	Alive
2	F/11	Stem cell	-/+	10	GVHD	GCV→ Oral VGCV	GCV 10 Oral VGCV*	319 Mutation: C603W	Dead**

D/R, donor/recipient; GCV, ganciclovir; GVHD, graft versus host disease; P, patient; PTx, posttransplant; Tx, transplant; VGCV, valganciclovir. *Adult: 900 mg/kg - day; pediatric: 30 mg/kg - day. **Because of bronchiolitis obliterans and pneumonia on day 380 posttransplantation.

recipients are at the risk of reactivation of a latent virus during immunosuppressive therapy, as well as of reinfection by a new virus strain hidden inside the allograft (12). Abundant data in the literature suggest that the resistance of CMV to GCV is associated with the lack of a therapeutic response and a progression to CMV disease (13). Some studies suggest that a high viral load, associated with the exposure to potentially inadequate levels of the drug, may increase the selection of drug-resistant mutants (14).

The present study identified a pediatric transplant patient with CMV infection as the first pediatric stem cell transplant recipient with UL97 mutation identified in Turkey. Pediatric recipients are at high risk for CMV infection after transplantation following primary infection, reactivation of latent CMV, or reinfection with a new strain (15). The incidence of GCV-resistant CMV infection has been reported to be 3.8% in pediatric allogeneic peripheral blood stem cell transplant recipients (16). The patient had Fanconi aplastic anemia, which might confer additional risk for persistent viremia leading to prolonged therapy and subsequent resistance.

One of the main risk factors for the emergence of resistance is D+/R- serostatus. However, resistance can also occur in seropositive organ recipients (17). Inadequate GCV doses can induce clinical resistance and favor the emergence of resistance-associated mutations within UL97 and, sometimes, UL54 (18).

Moreover, optimal ganciclovir doses can overcome virological resistance due to isolated UL97 resistance mutations, at least for a certain period (14).

The sequencing results of both cases are seen in figure 1.

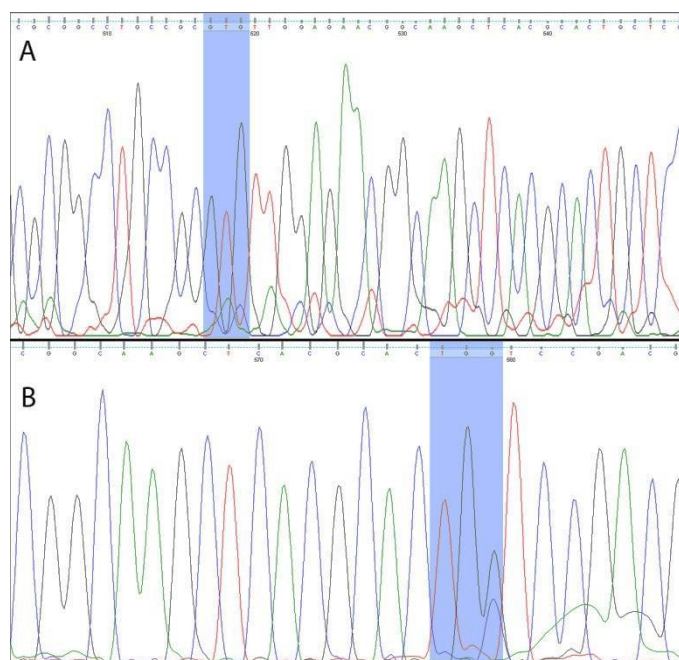


Figure 1: Sequencing results indicating mutations. In A, case 1, the light blue area is showing the A594V mutation, a C > T substitution of the nucleotide that resulted in Ala594Val. In B, case 2, the light blue area is showing the C603W mutation, a C > G substitution of the nucleotide that resulted in Cys603Trp.

Conclusions

Using GCV/VGCV for preemptive therapy, prophylaxis, treatment, and especially maintenance therapy for a long time with VGCV when CMV DNA is still detectable in peripheral blood might represent a risk factor for the emergence of CMV GCV resistance.

The present findings indicated that resistance to widely used GCV as therapy, preemptive therapy, and prophylaxis should be monitored carefully in transplant recipients routinely for good patient management and effective antiviral therapy.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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