Assessment of the Human Cytomegalovirus UL97 Gene to identify the Resistance to Ganciclovir in Kidney Transplant Recipients in Labbafi-Nejad Hospital

Shahnaz Sali,1 Farhad Soori,1 and Zahra Arab-Mazar1,2

1Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

2Corresponding author: Zahra Arab-Mazar, Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. E-mail: z.arabmazar@gmail.com

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Abstract

Background: Cytomegalovirus (CMV) is one of the most frequently encountered opportunistic viral pathogens in renal transplantation. Approximately, in 60% of the transplant recipients CMV infection can be observed and in > 20% symptomatic diseases can be developed. However, antiviral prophylaxis and treatment have reduced the CMV morbidity and mortality at the time of development of antiviral-resistance CMV strains that can significantly contributed to the adverse clinical outcomes in transplant recipients. Mutations in the human CMV UL97 kinase gene are a major mechanism of viral resistance to the anti-CMV drug “Ganciclovir (GCV)”. GCV, as the most widely used and recognized therapy for CMV, is a substrate for the UL97 kinase. Methods: The studied patients were renal transplant recipients in Tehran Labbafi Nejad hospital who were positive for CMV-DNA PCR test and have been treated with Ganciclovir. Patients who have been treated for at least 3 weeks with GCV and have not shown a proper therapeutic response were candidate for UL97 gene mutations associated with GCV resistance evaluation. Results: About 60 patients with positive CMV DNA PCR were hospitalized during one year study. Eventually, after 2 times measurement of CMV viral load at the end of the third week and third month of therapy with Ganciclovir, 5 cases were candidate for antiviral resistance evaluation. Genotypic testing was performed, but no mutation neither in UL97 nor in UL 54 was detected by the laboratory. Conclusions: The increasing use of antiviral drugs in transplant patients associated with the narrow range of antiviral agents effective to treat CMV have increased our need for further understanding of the risk factor for development of CMV antiviral resistance and it’s clinical impacts. Detection of UL97 gene mutation plays a major role to determine therapeutic strategies to treat patients infected with the resistant viruses.

Keywords: Cytomegalovirus, Ganciclovire, Drug Resistance Mutations, UL97 Gene

1. Background

Cytomegalovirus (CMV) is one of the major opportunistic especially after transplantation, and variant resistance to antiviral therapies. Human cytomegalovirus (HCMV) as one of the common virus, is epidemiologically varies in different regions of the world and between socioeconomic and age groups (1, 2). Therefore, for the accurate treatment of HCMV, diagnosis in an early stage is especially necessary to avoid poor clinical outcomes (3-5). Ganciclovir is a nucleoside analogue antiviral drug which is widely used to treat systemic CMV disease. To exert antiviral activity as an inhibitor of viral DNA polymerase (UL54), Ganciclovir should be phosphorylated. The UL97 kinase, a virus-encoded product, can activate the drug by monophosphorylation (6, 7). The CMV resistance to ganciclovir is favored by prolonged therapy and it is mainly associated with the presence of mutations within the UL97 gene (8, 9). As an impairment of GCV monophosphorylation, mutations in the human cytomegalovirus (HCMV) UL97 phosphotransferase have been associated with ganciclovir (GCV) resistance (8). Resistance to the GCV has been evaluated by many researchers in previous studies, while GCV have been used as the first-line treatment in most of HCMV patients (10).

During recent years, to detect HCMV-resistant mutations, many studied have been done and different methods such as the plaque reduction assay, ELISA, and DNA hybridization assays (11-13) have been used based on phenotypic testing, but these tests are burdensome, time-consuming, and labor-intensive (14). As a result, there is a significant need for early-stage detection of the emerging mutants (15).

The aim of this study was to determine the GCV-resistant HCMV in renal transplant recipient patients.

2. Methods

Our study focused on kidney transplant recipients with high levels of CMV viral load after antiviral therapy. 5 mL of the blood was collected from patients. After blood collection from patients, their sera were separated and stored at -80°C. DNA extraction was performed using the Roche DNA Mini kit, and it was extracted according to the manufacturer’s instructions. For checking the quality of sample, they were checked by a spectrophotometer (Nanodrop 2000), and they were kept at -80°C. Samples were detected and sent to the Labbafi Nejad specialty laboratory for identification of UL97 gene.
Specific regions of the UL97 gene of HCMV in the samples were amplified using nested PCR. The sequence of the forward primer in the first round of PCR was \(UL97-F1\) (5'-GGTTCATCAACAGAGTGGA-3') and the reverse primer was \(UL97-R1\) (5'-GGTTCCTCCTGGGACATAGTG-3'). The second round primers were \(UL97-F2\) (5'-TCACTCAACAGAGTGGA-3') and \(UL97-R2\) (5'-GGCACAGGACATCTGGGA-3'). The nested PCR was carried out in a 15 \(\mu L\) reaction mixture containing 7.5 \(\mu L\) master mix (Bie & Berntsen A/S), 1 \(\mu L\) of the equally-mixed primers, 1.5 \(\mu L\) DNA, and 5 \(\mu L\) ddH\(_2\)O. The following thermal cycling condition was used for PCR reaction: 94°C for 30 seconds, 54°C for 40 seconds, 72°C for 2 minutes for 40 cycles, and 72°C for 10 minutes.

All of the selected patients answered and completed questionnaires and informed consent forms for participation in this study.

3. Results

During the observation period, 60 recipients developed CMV infection with or without tissue-invasive diseases. The specific nested PCR primers amplified the mutation region in the \(UL97\) gene. The binding site of primers on the \(UL97\) gene of HCMV (270 bp) were nucleotide numbers 400 - 670. Unfortunately, there was no positive sample.

4. Discussion

Resistance of cytomegalovirus (CMV) to antiviral agents is the well-recognized phenomenon which has been observed in the laboratory and clinical environments (16, 17).

The emergence of drug-resistant HCMV strains especially in immunocompromised individuals with the active HCMV infection is a life-threatening condition (9). Drug-resistant HCMV was considered as a major problem in patients with acquired immunodeficiency syndrome until the recent introduction of highly active antiretroviral combination therapy, and unfortunately, we are faced a dramatically decrease in it’s the incidence in this clinical setting. Moreover, recently, HCMV antiviral drugs resistance in the transplantation is also known as an emerging problem. Ganciclovir-resistant CMV is mostly observed and caused clinical problems in solid-organ transplant recipients (18). One of the posttransplantation complications is the presence of CMV-seronegative recipients of the organs from seropositive donors. Ganciclovir therapy length before CMV resistance ranges from 51 to 438 days in the kidney transplant recipients (19). However, resistance was identified after 3 months of treatment for most patients (19-21).

\(UL97\) coding for a viral phosphotransferase and \(UL54\) coding for the viral DNA polymerase are two key viral genes which have been elucidated in the molecular mechanisms of drug-resistant HCMV and rely on the selection during treatment of HCMV strains harboring mutations. Generally, drug resistance mutations appear after the extended ganciclovir therapy. This most likely is due to the proofreading function of the viral DNA polymerase which greatly reduces nucleotide misincorporation resulting in a low mutation rates. The most prevalent sites of ganciclovir resistance mutations are in the \(UL97\) gene (22).

Approximately, 95% of the GCV-resistant HCMV strains contain one or more mutations in the \(UL97\) gene (23). Mutations in three specific codons (460, 594, and 595), have observed in approximately 70% of the GCV-resistant HCMV strains (24). The rate of HCMV drug resistance is widely vary depending on several factors such as type of patients, and several risk factors which have already been identified including the patient and disease-related factors, treatment-related factors, and viral factors (9, 25).

In the previous study some deletions in an immunocompromised patient associated with clinical resistance to the GCV, especially in the codons of the \(UL97\) gene have been reported. In another study, Hantz et al. explored a new GCV-resistance mutation in the kidney transplant patients which is a deletion of codon 601 of the \(UL97\) gene (6). Our study could not present any mutation in a GCV-resistant HCMV isolate among renal transplant recipients patients.

4.1. Conclusions

The present study demonstrates that we could not observe any mutation in patients in Labbafi-Nejad hospital. It is suggested that this mutation be investigated more using more cases and in a long period.

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Footnotes

Authors’ Contribution: Study concept and design: Dr. Shahnaz Sali; acquisition of data, analysis and interpretation of data: Farhad Soori; drafting of the manuscript: Zahra Arab-Mazar; study supervision: Dr. Shahnaz Sali.

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**References**


