Int J Infect. 2017 April; 4(2):e41189. doi: 10.5812/ij.-41189.

Published online 2016 November 8. **Research Article**

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-Mazar[1,*]

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

[*] *Corresponding 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

Received 2016 August 30; **Revised** 2016 September 05; **Accepted** 2016 September 05.

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 Labbafinejad hospital who were positive for CMV-DNA PCR test and have been treated with Ganciclovire. 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 Ganciclovire, 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 -20°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′-GTTTCATCACGACCAGTGGA-3′) and the reverse primer was *UL97*-R1 (5′-GGTCCTCCTCGCAGATTATG-3′). The second round primers were *UL97*-F2 (5′-TCATCACGACCAGTGGAAGCT-3′) and *UL97*-R2 (5′-GCGACACGAGGACATCTTGG-3′). The nested PCR was carried out in a 15 μL reaction mixture containing 7.5 μL master mix (Bie & Berntsen A/S), 1 μL of the equally-mixed primers, 1.5 μL DNA, and 5 μL ddH_2O. 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 resistance and it is predominantly observed among 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 Labbaffi-Nejad hospital. It is suggested that this mutation be investigated more using more cases and in a long period.

Acknowledgments

The authors thank the staff of Labbafi-Nejad hospital laboratory for sample collection. This Article has been extracted from the thesis written by Dr. Soori in school of Medicine, Shahid Beheshti University of Medical Sciences.

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.

Conflict of Interest: There is no conflict of interest regarding the publication of this paper.

Funding/Support: This work was financially supported by the infectious diseases and tropical medicine research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

1. Luganini A, Caposio P, Mondini M, Landolfo S, Gribaudo G. New cell-based indicator assays for the detection of human cytomegalovirus infection and screening of inhibitors of viral immediate-early 2 protein activity. *J Appl Microbiol.* 2008;**105**(6):1791–801. doi: 10.1111/j.1365-2672.2008.03927.x. [PubMed: 19120629].
2. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol.* 2010;**20**(4):202–13. doi: 10.1002/rmv.655. [PubMed: 20564615].
3. Numata S, Nakamura Y, Imamura Y, Honda J, Momosaki S, Kojiro M. Rapid quantitative analysis of human cytomegalovirus DNA by the real-time polymerase chain reaction method. *Arch Pathol Lab Med.* 2005;**129**(2):200–4. doi: 10.1043/1543-2165(2005)129<200:RQAOHC>2.0.CO;2. [PubMed: 15679421].
4. Boriskin YS, Fuller K, Powles RL, Vipond IB, Rice PS, Booth JC, et al. Early detection of cytomegalovirus (CMV) infection in bone marrow transplant patients by reverse transcription-PCR for CMV spliced late gene UL21.5: a two site evaluation. *J Clin Virol.* 2002;**24**(1-2):13–23. [PubMed: 11744424].
5. Sanchez JL, Storch GA. Multiplex, quantitative, real-time PCR assay for cytomegalovirus and human DNA. *J Clin Microbiol.* 2002;**40**(7):2381–6. [PubMed: 12089251].
6. Hantz S, Michel D, Fillet AM, Guigonis V, Champier G, Mazeron MC, et al. Early selection of a new UL97 mutant with a severe defect of ganciclovir phosphorylation after valaciclovir prophylaxis and short-term ganciclovir therapy in a renal transplant recipient. *Antimicrob Agents Chemother.* 2005;**49**(4):1580–3. doi: 10.1128/AAC.49.4.1580-1583.2005. [PubMed: 15793144].
7. Baldanti F, Silini E, Sarasini A, Talarico CL, Stanat SC, Biron KK, et al. A three-nucleotide deletion in the UL97 open reading frame is responsible for the ganciclovir resistance of a human cytomegalovirus clinical isolate. *J Virol.* 1995;**69**(2):796–800. [PubMed: 7815545].
8. Baldanti F, Michel D, Simoncini L, Heuschmid M, Zimmermann A, Minisini R, et al. Mutations in the UL97 ORF of ganciclovir-resistant clinical cytomegalovirus isolates differentially affect GCV phosphorylation as determined in a recombinant vaccinia virus system. *Antiviral Res.* 2002;**54**(1):59–67. [PubMed: 11888658].
9. Baldanti F, Lurain N, Gerna G. Clinical and biologic aspects of human cytomegalovirus resistance to antiviral drugs. *Hum Immunol.* 2004;**65**(5):403–9. doi: 10.1016/j.humimm.2004.02.007. [PubMed: 15172438].
10. Hantz S, Garnier-Geoffroy F, Mazeron MC, Garrigue I, Merville P, Mengelle C, et al. Drug-resistant cytomegalovirus in transplant recipients: a French cohort study. *J Antimicrob Chemother.* 2010;**65**(12):2628–40. doi: 10.1093/jac/dkq368. [PubMed: 20961907].
11. Prix L, Maierl J, Jahn G, Hamprecht K. A simplified assay for screening of drug resistance of cell-associated cytomegalovirus strains. *J Clin Virol.* 1998;**11**(1):29–37. [PubMed: 9784141].
12. Pepin JM, Simon F, Dussault A, Collin G, Dazza MC, Brun-Vezinet F. Rapid determination of human cytomegalovirus susceptibility to ganciclovir directly from clinical specimen primocultures. *J Clin Microbiol.* 1992;**30**(11):2917–20. [PubMed: 1333484].
13. Gerna G, Sarasini A, Percivalle E, Zavattoni M, Baldanti F, Revello MG. Rapid screening for resistance to ganciclovir and foscarnet of primary isolates of human cytomegalovirus from culture-positive blood samples. *J Clin Microbiol.* 1995;**33**(3):738–41. [PubMed: 7751388].
14. Landry ML, Stanat S, Biron K, Brambilla D, Britt W, Jokela J, et al. A standardized plaque reduction assay for determination of drug susceptibilities of cytomegalovirus clinical isolates. *Antimicrob Agents Chemother.* 2000;**44**(3):688–92. [PubMed: 10681339].
15. Chakravarti A, Kashyap B, Matlani M. Cytomegalovirus infection: an Indian perspective. *Indian J Med Microbiol.* 2009;**27**(1):3–11. [PubMed: 19172051].
16. Erice A. Resistance of human cytomegalovirus to antiviral drugs. *Clin Microbiol Rev.* 1999;**12**(2):286–97. [PubMed: 10194460].
17. Goryainov VA, Kaabak MM, Babenko NN, Platova EN, Aganesov AG, Morozova MM, et al. [Resistant cytomegalovirus infection in related donor kidney allograft recipients]. *Ter Arkh.* 2016;**88**(6):73–6. [PubMed: 27296265].
18. Hanson KE, Swaminathan S. Cytomegalovirus antiviral drug resistance: future prospects for prevention, detection and management. *Future Microbiol.* 2015;**10**(10):1545–8. doi: 10.2217/fmb.15.82. [PubMed: 26437628].
19. Lurain NS, Bhorade SM, Pursell KJ, Avery RK, Yeldandi VV, Isada CM, et al. Analysis and characterization of antiviral drug-resistant cytomegalovirus isolates from solid organ transplant recipients. *J Infect Dis.* 2002;**186**(6):760–8. doi: 10.1086/342844. [PubMed: 12198609].
20. Limaye AP. Ganciclovir-resistant cytomegalovirus in organ transplant recipients. *Clin Infect Dis.* 2002;**35**(7):866–72. doi: 10.1086/342385. [PubMed: 12228824].
21. Lowance D, Neumayer HH, Legendre CM, Squifflet JP, Kovarik J, Brennan PJ, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. *N Engl J Med.* 1999;**340**(19):1462–70. doi: 10.1056/NEJM199905133401903. [PubMed: 10320384].
22. Avery RK, Arav-Boger R, Marr KA, Kraus E, Shoham S, Lees L, et al. Outcomes in Transplant Recipients Treated With Foscarnet for Ganciclovir-Resistant or Refractory Cytomegalovirus Infection. *Transplantation.* 2016;**100**(10):e74–80. doi: 10.1097/TP.0000000000001418. [PubMed: 27495775].
23. Chou S. Cytomegalovirus UL97 mutations in the era of ganciclovir and maribavir. *Rev Med Virol.* 2008;**18**(4):233–46. doi: 10.1002/rmv.574. [PubMed: 18383425].
24. Komatsu TE, Pikis A, Naeger LK, Harrington PR. Resistance of human cytomegalovirus to ganciclovir/valganciclovir: a comprehensive review of putative resistance pathways. *Antiviral Res.* 2014;**101**:12–25. doi: 10.1016/j.antiviral.2013.10.011. [PubMed: 24184129].
25. Ayala E, Greene J, Sandin R, Perkins J, Field T, Tate C, et al. Valganciclovir is safe and effective as pre-emptive therapy for CMV infection in allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2006;**37**(9):851–6. doi: 10.1038/sj.bmt.1705341. [PubMed: 16532016].