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Original Article

بررسی موتاسیون‌های ژن *UL54* مقاومت علیه گانسیکلوویر در گیرندگان پیوند کلیه و سلول‌های بنیادی خونساز مبتلا به عفونت سیتومگالوویروس

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چکیده

سابقه و هدف: مقاومت سیتومگالوویروس به گانسیکلوویر با جهش‌های خاص در ژن *UL54* عاملی در شکست درمان و پیشرفت بیماری در گیرندگان پیوند اعضا، به‌ویژه پیوند کلیه و سلول‌های بنیادی خونساز است. این مطالعه با هدف تعیین جهش‌های منجر به مقاومت در ژن *UL54* سیتو مگالوویروس انسانی انجام شد.

مواد و روش‌ها: در این مطالعه پس از غربالگری ۲۳ گیرنده پیوند کلیه و ۲ گیرنده سلول‌های بنیادی خونساز با تست CMV اولیه مثبت، ٦ بیمار بر اساس معیارهای ورود (حداقل ۲ پیگیری با نتایج Real-time PCR مثبت CMV از پیگیری دوم به بعد) وارد مطالعه نهایی شدند. ژن *UL54* با Nested-PCR تکثیر شد و محصولات PCR برای توالی‌یابی با استفاده از روش سنگر تعیین توالی شدند. برای تجزیه و تحلیل نتایج توالی‌یابی از نرم افزار فینچ (نسخه ۱/٤/۰) استفاده شد.

یافته‌ها: پس از بررسی نتایج توالی‌یابی، هیچ جهش شناخته شده‌ای در ٤ بیمار دریافت‌کننده پیوند کلیه مشاهده نشد. همچنین، جهش serine 882 insertion در ژن *UL54* در ۱ بیمار گیرنده پیوند سلول‌های بنیادی خونساز مشاهده شد. بررسی درخت فیلوژنی ژن *UL54* نشان داد که جدایه ایرانی از نظر اجدادی به ۲۰ سویه مرجع از جمله سویه مرلین تعلق دارد.

نتیجه‌گیری: با توجه به اینکه ظهور جهش serine 882 insertion می‌تواند پتانسیل درمان را ضعیف کند و پاسخ به گانسیکلوویر را دچار مشکل کند، نظارت بر بیماران مورد نظر، تعیین بار ویروسی و ارزیابی پاسخ یا عدم پاسخ آن‌ها به درمان بسیار مهم است.

واژگان کلیدی: پیوند کلیه، پیوند سلول‌های بنیادی خونساز، سیتومگالوویروس، مقاومت دارویی.

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Original Article

Study of the ganciclovir resistant *UL54* gene mutations in cytomegalovirus infected Kidney and Hematopoietic stem cell transplant recipients

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Abstract

Background & Objectives: Cytomegalovirus resistance to ganciclovir with specific mutations in the *UL54* gene is a factor in treatment failure and disease progression in organ transplant recipients, particularly kidney and hematopoietic stem cell transplant recipients. This study aimed to determine the mutations leading to resistance in the *UL54* gene of human cytomegalovirus.

Materials & Methods: In this study, after screening 23 kidney transplant and 2 hematopoietic stem cell transplant recipients with a positive initial CMV test, 6 patients were included in the final study based on the inclusion criteria (at least 2 available follow-ups with positive CMV Real-time PCR results). The *UL54* gene was amplified using Nested-PCR, and the PCR products were then sequenced using the Sanger sequencing method. Finch software (version 1.4.0) was used to analyze the sequencing results.

Results: After reviewing the sequencing results, no known mutations were observed in 4 kidney transplant recipients. Also, a serine 882 insertion mutation in the *UL54* gene was observed in 1 hematopoietic stem cell transplant recipient. Examination of the phylogenetic tree of the *UL54* gene showed that the Iranian isolate ancestrally belongs to 20 reference strains, including the Merlin strain.

Conclusion: Given that the emergence of the serine 882 insertion mutation can weaken the potential for treatment and impair response to ganciclovir, it is very important to monitor the patients in question, determine the viral load, and assess their response or lack of response to treatment.

Keywords: Kidney transplantation, Hematopoietic stem cell transplantation, Cytomegalovirus, Drug resistance.

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Introduction

Cytomegalovirus or human herpesvirus 5 is a member of the Herpesviridae family, which can cause severe disease in transplant recipients due to its ubiquitous presence (1). Saliva, urine, sexual contact, placental transfer, breastfeeding, blood transfusion, solid organ or hematopoietic stem cell transplantation are the main routes of spread of human cytomegalovirus (2).

It is hypothesized that cytomegalovirus

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infection in Iranian kidney and hematopoietic stem cell transplant recipients treated with ganciclovir may cause alterations in the *UL54* gene, and the pattern of these alterations in Iranian strains could differ from those in other regions (3).

Cytomegalovirus (CMV) usually causes asymptomatic infections in immunocompetent hosts, but among transplant recipients, it causes fatal diseases including pneumonia, enteritis, cystitis, and encephalitis (4). This infection is controllable in such patients and is latent before the appearance of clinical symptoms (5). After initial infection, CMV has a subclinical, lifelong latent infection in the myeloid lineage cells derived from the Hematopoietic Stem Cell Transplant (6). In solid organ transplant recipients, one of the main causes of infection and disease is cytomegalovirus, which affects between 50% and 90% of kidney allograft recipients, and 8% to 32% of these infections are related to CMV disease (7). Kidney transplant recipients (KTRs) are more susceptible to opportunistic infections, which increases the risk of transplant rejection, higher incidence of chronic allograft nephropathy, and increased patient mortality (8). After transplantation, Monitoring the severity of CMV infectivity is done by determining the viral load using qPCR (9). During the same period, antiviral therapy, including ganciclovir, valganciclovir, foscarnet, cidofovir, letermovir, and maribavir are prescribed (10). A key challenge in managing CMV infection in transplant recipients is ganciclovir resistance, which arises from genetic mutations in the *UL54* gene (11). Using oral valganciclovir as prophylactic or preemptive therapy, the incidence of late *CMV* disease and its mortality after HSCT are reduced by 5% and 17%, respectively (12). After lifelong incubation in immunocompetent and immunocompromised individuals (2), viral reactivation occurs by lytic virus phase (2). With the introduction of ganciclovir in the mid-1980s, the first antiviral combination against cytomegalovirus (CMV) resistance has become a growing problem in transplant recipients (13). In solid organ transplant recipients, the range of CMV resistance to antiviral drugs ranges from 0.4% to 11.9% and 1 to 5% in patients who have undergone hematopoietic stem cell transplantation (14,15). Currently, ganciclovir and valganciclovir are the first-line treatments for CMV disease in kidney transplant recipients, and their use has resulted in a reduction in CMV disease and related morbidity in solid organ transplant recipients, although this may contribute to the development of drug resistance (16). However, the extensive and prolonged use of antiviral drugs for CMV prophylaxis, along with the use of immunosuppressive medications, have contributed to the emergence of antiviral-resistant CMV strains and the progression of the disease (17). Drug-resistant HCMV infection in allogeneic hematopoietic cell transplant (HCT) recipients can be life-threatening and fatal. Therefore, continuous monitoring of viral load and determination of the severity of infectivity are necessary (18).

Typically, sequence analysis is one of the genotyping methods used to detect mutations (19). However, most of these assays are limited to detecting known mutations. Given the success of the Nested PCR method in several studies for identifying mutations in human cytomegalovirus, its use in our study is also considered advantageous and superior (20). In addition, Sanger sequencing is the gold standard for detecting ganciclovir-resistant

mutations in *UL54* gene (21).

The aim of this study is to identify the pattern of clinical mutations in the cytomegalovirus *UL54* gene following ganciclovir treatment, and to evaluate the impact of these mutations on disease progression in kidney and hematopoietic stem cell transplant recipients infected with Iranian CMV strains.

Materials and methods

A: Study patients and samples: In this study, after screening 23 kidney transplant recipients and 2 hematopoietic stem cell transplant recipients with a positive initial CMV test, 6 patients (4 kidney transplant recipients and 2 hematopoietic stem cell transplant recipients) were included in the final study based on inclusion criteria, having at least 2 follow-ups with positive CMV Real-time PCR results (viral load ≥ 10000 copies/ml) from the second follow-up onwards. The remaining 19 kidney transplant patients were excluded from the study. The study population of CMV-positive kidney transplant recipients consisted of 100% males (4/4) and the average age was 45.00 ± 11.52 (mean ± SD) and CMV-positive Hematopoietic Stem Cell transplant recipients consisted of 50.0% males (1/2) and 50.0% females (1/2), and the average age was 27.5 ± 2.12 (mean ± SD) who underwent organ transplants in the organ transplant departments of Abu Ali Sina and Namazi Shiraz hospitals between 2015 and 2017. A total of six specimens from 6 patients that had an amplifiable HCMV *UL54* gene were selected for sequencing of the *UL54* gene using the Sanger method. To conduct this study, the code of ethics was first received from the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1401.734), and written informed consents were provided by all participants.

B: CMV nucleic acid extraction and measurement of viral load: The DNP™ kit (Cinagene Company, Iran) was used to extract nucleic acid from plasma (22,23) and then using the Real-time PCR kit (Gene Proof company, Czech Republic) and using the quantitative TaqMan Real-Time PCR method, the presence of infection and the quantitative viral load were measured (24,25). The master mix was prepared to a total volume of 21 µL, comprising 5 µL of extracted DNA, 15 µL of PCR mix, and 1 µL of internal control (IC) gene. The temperature program for the Real-time PCR reaction consisted of 1 cycle of 10 minutes at 95°C for pre-denaturation, followed by 45 cycles with three steps: 5 seconds at 95°C for denaturation, 40 seconds at 60°C for annealing, and 20 seconds at 72°C for extension. The reactions were carried out using the 7900HT Real-time ABI PCR system (Applied Biosystems, USA) (26).

C: Primer selection: Primer design was done using Allele ID7, Primer Blast and Gene Runner software. They were then checked using Nucleotide Blast and Oligo software and sent to Tekapo for synthesis (27).

D: Investigating the changes of UL54 gene and Sequencing: To investigate genetic changes in the *UL54* (3729-pb) gene, all samples were examined using the Nested-PCR method and the desired fragment was isolated for sequencing. To perform this step, specifically designed primers were used, four pairs of primers were designed for the *UL54* gene (28). The sequence of the first round and second round primers, materials used and thermal program of the thermocycler is shown in Table 1. The PCR samples were electrophoresed using a 1.5% agarose gel containing TAE buffer (1x). The gel was then transferred to a

UV illuminator and the bands were observed using UV light. After performing the steps of DNA extraction from agarose gel, these samples were sent to Maxogen, South Korea, for sequencing and bioinformatics evaluations using the Sanger method and sequenced using primers specific to this region (28). The results of this step were analyzed using specialized software, including Finch and Blast (29).

Table 1: Primers used for simple and nested PCR of UL54 gene, F, forward; R, reverse.

Gene	Method of PCR	Primer sequence	Materials and concentration required for simple and nested PCR steps			Thermal program of the thermocycler
UL54	Simple	F:5' GTCTACGAGTTCCCTTCCG R:5' GCATTAGCCACGAAACAAC		Simple PCR	Nested PCR	
UL54	Nested	F:5' GCTGCTGCTGGGCTTTA R:5' GCATTAGCCACGAAACAAC	Buffer 10 ×	2.5 µL	2.5 µL	
UL54	Simple	F:5' GTTGCGGCGTGTCATCTTTG R:5' CAGGGTGGAGTAGCAGAGGT	dNTP 10 nM	0.75 µL	0.75 µL	
UL54	Nested	F:5' GTCACCTAACGCCGCTATCA R:5' GGGTAGAGGCTGGCAAAGTC	Mgcl2 50 mM	0.75 µL	0.75 µL	1cycle: 95°C, 5min 40cycles: 94°C, 1min; 55° C, 30sec; 72°C, 1min 1cycle: 72° C, 5min
UL54	Simple	F:5' GGCTCACAACCTCTGCTACTC R:5'GCAAAAAACACGGCTCTGAA	Taq DNA Polymerase 5 unit/µl	0.25 µL	0.25 µL	
UL54	Nested	F:5' TACCCCGTGGACCCTGC R:5' GCAAAAAACACGGCTCTGAA				
UL54	Simple	F:5' GCGGGAGGGGGATTCGG R:5' TCAAAGAGCAGCGAGAGGAC	Forward and Reverse primers 10 µM	1 µ	1 µ	
UL54	Nested	F:5' GCGGGAGGGGGATTCGG	Distilled deionized water	14.75 µl	15.75 µl	
		R:5' TGACGCCCTTGACGAACTC	Template DNA	4 µl	3 µl	

E: Analysis of sequencing results: The nucleotide sequences were compared with the Merlin strain sequence. To analyze the PCR products, the sequencing results were checked using Finch software (version 1.4.0), nucleotide blast, and alignment, and then the mutated sequence was registered in the Gene Bank to receive an accession number.

To draw a phylogenetic tree of the sequences with mutations, alignment was performed using the ClustalW alignment tool in MEGA X software (version 10.0.5), and then the phylogenetic tree was drawn using the Maximum Likelihood method and Tamura-Nei model, and using the bootstrap test with 1,000 replicates (29).

F: Statistical calculations: Statistical analyses were conducted using SPSS version 26 software. The Mann-Whitney U test, a non-parametric method, was applied to compare the mean viral loads between the two groups (kidney transplant recipients and hematopoietic stem cell transplant recipients). The Chi-square test was used to assess the association between mutation type and variables such as age, sex, blood group,

underlying conditions, and laboratory factors including hemoglobin, white blood cells, platelet, creatinine, sodium, and potassium. Spearman's correlation coefficient analysis (two-tailed) was used to determine the relationship between mean viral loads and treatment duration in both patient groups. A p-value of less than 0.05 was considered statistically significant (24).

Results

A: Demographic data of CMV *DNAmia patients:* After performing PCR using specific primers for *UL54* gene, a total of 4 samples from 4 patients (4 males; 100%) kidney transplant recipients had a mean age of 45.00 11.52 ± (range, 35-60 years) with a range of 1.52×10^4 to 2.072×10^6 copies/mL and 2 samples from 2 patients (1 females; 50% and 1 males; 50%) Hematopoietic Stem Cell Transplant recipients had a mean age of 27.50 ± 2.12 (range, 26,29 years) with a range of 3.07×10^5 to 6.00×10^5 copies/mL were identified as potentially harboring mutations in the *UL54* gene and were subsequently sent to Maxogen Company in South Korea for sequencing analysis. None of the patients were treated with valganciclovir before confirming a positive result for CMV infection. During the development of resistance, all patients were treated with valganciclovir (Valcyte: 900 mg per day). The demographic characteristics of the 6 HCMV DNAmia patients is shown in Table 2.

Table 2: Characteristics of 6 kidney and Hematopoietic Stem Cell Transplant recipients.

Patient NO.	Sex	Age (yr.)	Type of transplantation	Underlying disease	*UL54* gene mutations	Viremia peak copies/ mL	Duration of treatment (day)	Patients living status (Death or Alive)
1	Male	37	Kidney	ESRD	No mutation	218000	7	Alive
2	Male	60	Kidney	ESRD	No mutation	15200	210	Alive
3	Male	35	Kidney	HTN	No mutation	1000000	300	Alive
4	Male	48	Kidney	HTN	No mutation	207200	14	Alive
5	Male	29	Hematopoietic Stem Cell	ALL	serine-882 insertion	307000	23	Alive
6	Female	26	Hematopoietic Stem Cell	ALL	No mutation	600000	210	Alive

NOTE. No, number; ESRD, End-Stage Renal Disease; HTN, Hypertension; ALL, Acute lymphoblastic leukemia; Neg, negative; Pos, positive.

B: Investigation of Hematopoietic Stem Cell Transplant recipient patient with valganciclovir resistance mutation: A 29-year-old man with acute lymphoblastic leukemia (ALL) received his father's Hematopoietic Stem Cell Transplant. This patient had a CMV DNA viral load of 307,000 copies/ml on day 90 after CMV transplantation and before treatment. During follow-up, while the patient was using VGCV (Valcyte: 900 mg per day), CMV DNA became positive (166,000 copies/mL). After checking the sequencing results of the *UL54* gene, on the 90th day after transplantation with a viral load of 307,000 copies/ml, the serine 882 insertion mutation was found to be resistant to GCV. The viral load in the patient's blood was measured once a week after using valganciclovir, and CMV DNA levels

decreased 23 days after valganciclovir treatment. The location of the serine 882 insertion mutation in the *UL54* gene is shown in Figure 1.

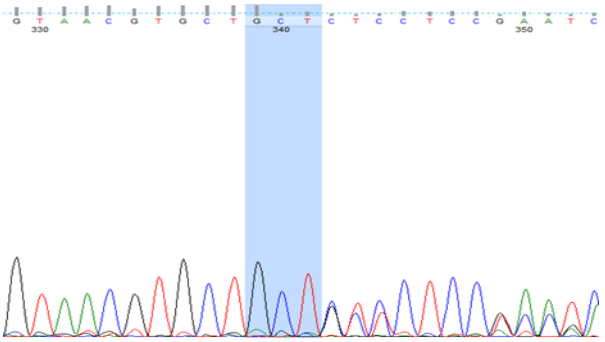

Fig 1: Site of serine 882 insertion mutation in HCMV *UL54* gene.

C: Sequencing results: After of reviewed of sequencing 6 HCMV DNAmia patients (4 patients of Kidney transplant recipients and 2 patients Hematopoietic Stem Cell Transplant recipients) no known mutations conferring ganciclovir resistance in *UL54* gene in the former recipients. However, we detected serine 882 insertion mutation (50.0%) in *UL54* gene which known conferring ganciclovir resistance in 1 patient (50.0%) Hematopoietic Stem Cell Transplant recipient. Emergence of this mutation was associated with increased viral load, indicating that this mutation confers resistance to GCV.

D: Statistical analysis: No significant difference was found in the mean viral loads between the two study groups (kidney and hematopoietic stem cell transplant recipients), with a p-value = 0.32. Additionally, no significant association was observed between the type of mutation and risk factors such as mean age, gender, blood group, underlying diseases, and laboratory parameters, with a p-value = 1.000. Also, there was no significant relationship between the mean viral load and duration of treatment in both groups, with a p-value = 0.67 .

E: Phylogenetic analysis of Iranian strains: A phylogenetic tree of the *UL54* gene was constructed to examine the genetic diversity and relationships between this study isolate and reference strains for comparison.

This analysis involved 20 nucleotide sequences. Accession number of nucleotide sequence with mutations were obtained after registration in the GenBank database.

The resultant accession number MZ723793 of the *UL54* gene was aligned with 20 HCMV strain sequences in GenBank with MEGA X software (version 10.0.5) (http://www.megasoftware.net) and was used to construct a phylogenetic tree with 1,000 bootstrap replicates. The alignment of this sequence with twenty reference sequences is shown in Figure 2. Phylogenetic analysis showed that the Iranian isolates belong to 20 reference strains in terms of ancestry (Figure 3). Also, no intraspecific differences were observed between isolates. According to the evolutionary distance table, accession number MZ723793 has the smallest evolutionary distance to the reference strains (Figure 4).

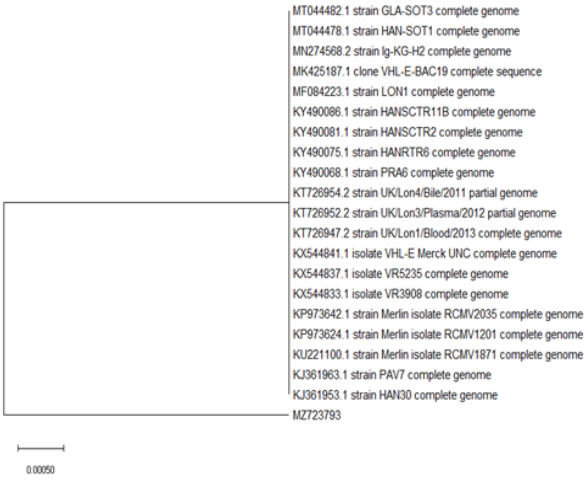

Fig 3: Phylogeny tree based on isolates from hematopoietic stem cell transplant recipient.

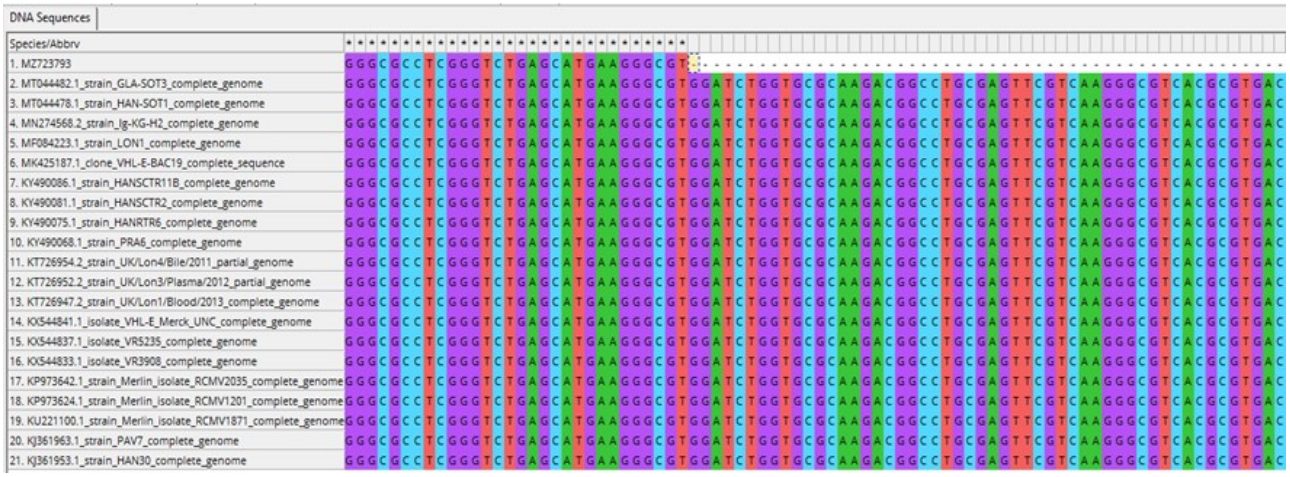

Fig 2: Alignment of human *cytomegalovirus* strain with reference strains.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. MZ723793																	
2. MT044482.1 strain GLA-SOT3 complete genome	0.006																
3. MT044478.1 strain HAN-SOT1 complete genome	0.006	0.000															
4. MN274568.2 strain lg-KG-H2 complete genome	0.006	0.000	0.000														
5. MK425187.1 clone VHL-E-BAC19 complete sequence	0.006	0.000	0.000	0.000													
6. MF084223.1 strain LON1 complete genome	0.006	0.000	0.000	0.000	0.000												
7. KY490086.1 strain HANSCTR11B complete genome	0.006	0.000	0.000	0.000	0.000	0.000											
8. KY490081.1 strain HANSCTR2 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000										
9. KY490075.1 strain HANRTR6 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
10. KY490068.1 strain PRA6 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
11. KT726954.2 strain UK/Lon4/Bile/2011 partial genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
12. KT726952.2 strain UK/Lon3/Plasma/2012 partial genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
13. KT726947.2 strain UK/Lon1/Blood/2013 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
14. KX544841.1 isolate VHL-E Merck UNC complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
15. KX544837.1 isolate VR5235 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
16. KX544833.1 isolate VR3908 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
17. KP973642.1 strain Merlin isolate RCMV2035 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
18. KP973624.1 strain Merlin isolate RCMV1201 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
19. KU221100.1 strain Merlin isolate RCMV1871 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20. KJ361963.1 strain PAV7 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
21. KJ361953.1 strain HAN30 complete genome	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Fig 4: Evolutionary distance of the Iranian strain obtained from a hematopoietic stem cell transplant recipient with reference strains.

Discussion

In patients with weakened immune system, particularly in Hematopoietic Stem Cell or solid organ transplants, human cytomegalovirus (HCMV) causes serious complications. To decrease mortality and morbidity in transplant recipients, anti-CMV prophylactic treatment is used early in the transplant. However, the emergence of drug-resistant cytomegaloviruses has increased due to the increased use of anti-cytomegalovirus drugs. Although drug resistance depends on the transplanted organ and immune-suppressing regimen, it is usually seen in 5 to 10% of cases in patients who are negative for the virus and receive it from a positive donor (D+/R-) (30). Mutations in the *UL54* gene cause drug resistance. CMV drug resistance is detected using clinical genotypic tests (31).

Ganciclovir resistance is linked to mutations in one or both of the *UL54* and *UL97* genes of CMV. In some cases, resistance mutations in the *UL54* gene are usually associated with mutations in *UL97* gene. However, in many cases only *UL54* gene mutations have been reported. Mutations that cause resistance in the *UL54* gene typically occur over a broad region (between codons 300 and 1000) and can lead to

resistance to one or more drugs (32). All cases with *UL54* gene mutations alone were related to BMT and exhibited a prolonged treatment history with GCV. A previous study reported that three Hematopoietic Stem Cell Transplant recipients treated with GCV and FOS had mutations only in the *UL54* gene (33).

The objective of this study is to investigate the possibility of mutations associated with drug resistance in Iranian strains *UL54* gene after treatment in kidney and hematopoietic stem cell transplant recipients. In this study, viral DNA in the blood of 6 patients after kidney and hematopoietic stem cell transplantation with cytomegalovirus infection was analyzed to determine the incidence of ganciclovir resistance and detect mutations in the *UL54* gene.

Also, six patients failed to respond to antiviral treatment despite treatment with ganciclovir, and their viral load did not show a significant reduction. Analysis of the DNA sequences of cytomegalovirus strains obtained from kidney transplant recipients before and after treatment showed no significant mutations in the *UL54* gene, although 1 mutation leading to resistance was observed in one hematopoietic stem cell transplant recipient.

From the 25 patients included in our study, 6 samples from 6 patients (4 kidney transplant recipients and 2 hematopoietic stem cell transplant recipients) were chosen for genotypic analysis based on a positive PCR result for CMV in plasma.

In recent years, there has been growing concern regarding the emergence of drug-resistant cytomegalovirus strains in severely immunosuppressed transplant recipients. The presence of these resistant strains has been observed in both solid organ and hematopoietic stem cell transplant patients as well (34).

Hall Sedlak et al. identified the V715M and N408D mutations in the *UL54* gene in 3 out of 41 patients tested who had *UL54* resistance mutations (35). Another study by Baldanti et al. in 1996 reported that the Pro628-Leu, Ser655-Leu, Asn685-Ser, Thr700-Ala, Ser885-insertion, and Ala886-Thr substitutions confer foscarnet resistance in the *UL54* gene of clinical isolates from AIDS patients (36). In a 2007 study conducted by Scott et al., two mutations, A834P and N408K, in the *UL54* gene were separately detected in kidney and lung transplant recipients, while a third mutation, L737M, was identified in a liver transplant recipient. The N408K mutation was found to confer resistance to ganciclovir and cidofovir, while the A834P mutation was associated with resistance to ganciclovir, cidofovir, and foscarnet (19). In a 2013 study conducted by Daikoku et al. on 13 clinical samples from 7 bone marrow and kidney transplant recipients, the V355A and A688V substitutions in the *UL54* gene were identified in most of the patients (37). In a 2014 study conducted by Cho et al., they showed that the exonuclease substitutions D413N, K513N, and D539G confer resistance to cidofovir and ganciclovir, while the C773G mutation confers resistance to foscarnet, and the C607V mutation confers resistance to ganciclovir (38). In a 2021 study by Yang et al., conducted on 112 patients treated for *cytomegalovirus* infection, 12 novel mutations were discovered in the *UL54* gene. These mutations included M827I, P342S, S384F, K434R, S673F, T754M, R778H, C814S, G878E, S976N, E888K, and S880L (39). In a 2023 study conducted by Resio et al. on 108 plasma samples from 96 transplant recipients suspected of having antiviral-resistant *cytomegalovirus*, the T503I

mutation was found to be the most prevalent in the *UL54* gene, occurring in 3 out of 7 patients (29). The results of these studies, similar to the present study, highlight the impact of mutations in the *UL54* gene on ganciclovir resistance.

The results of the present study are consistent with those of Homar et al., who showed that delayed viral clearance is not necessarily associated with cytomegalovirus drug resistance (40). Van der Beek et al. also demonstrated that in kidney transplant recipients treated with VGCV, resistance is infrequently observed and has minimal impact on treatment failure (41). In a 2010 study of transplant recipients, a CMV-positive kidney transplant recipient was found to have the *UL54* A834P mutation, which conferred resistance to ganciclovir, cidofovir, and foscarnet, 158 days after antiviral treatment (42). Although only 6 samples from 6 patients were tested in the present study, it is reasonable to assume that other patients with consistently negative PCR results do not harbor ganciclovir -resistant strains. The *UL54* gene mutation (serine 882 insertion) identified in the present study has been previously reported to occur in cytomegalovirus-susceptible isolates (43). It should be noted that *UL54* gene mutations predispose human *cytomegalovirus* to drug resistance. Therefore, continuous monitoring of human cytomegalovirus in Iran on Iranian strains is essential to understand the status of antiviral resistance.

The population in present study had several limitations due to the small number of patients with resistant cytomegalovirus infection. In addition, these patients were clinically suspected of having drug-resistant cytomegalovirus infection.

In a 2019 study by Alwan et al. on symptomatic infants in Iraq, three predominant genotypes, gB1, gB2, and gB3, were identified in infants and children infected with HCMV (44). In a 2018 study by Mousavi et al. on cervix-isolated samples, it was observed that the genome sequence of the HCMV-*DB* strain was similar to that of the Toledo strain, which was initially isolated from a child's urine sample (45). In a study by Fang et al. in 2010 on children who were recipients of renal and hematopoietic stem cell transplantation (HSCT), examination of the phylogenetic tree of the *UL97* gene showed that most polymorphisms belonged to the AD169 strain, with cluster 1 from children, cluster 2 from HSCT recipients, and 13 sequences from kidney transplant recipients in clusters 1 and 2 (46).

In this study, the *UL54* DNA sequence with accession number MZ723793 of the HCMV Iranian strain showed equally belonged to 20 reference strains in terms of ancestry including the Merlin strain.

Conclusion

This study was conducted to determine ganciclovir resistance mutations in the *UL54* gene of *cytomegalovirus*. The results showed that in a hematopoietic stem cell transplant recipient, strains with a mutation (serine 882 insertion) in the *UL54* gene could have the potential to be resistant to ganciclovir. Also, no mutations were observed in kidney transplant recipients, need future completed studies.

Ethical Considerations

The authors of this article have adhered to all ethical principles, including avoiding plagiarism, upholding literary standards, refraining from simultaneous publication, and abstaining from data manipulation and fabrication.

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Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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