

Assessment of Human Cytomegalovirus Viral Load in Kidney Transplant Recipients in Tehran, Iran

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ABSTRACT

Background: The purpose of this study is to evaluate the viral load of active human cytomegalovirus (HCMV) infection in the plasma samples of people suspected of kidney transplant rejection and to investigate the host and risk factors related to the activation of HCMV in these patients.

Materials & Methods: This cross-sectional study was conducted between December 2022 and June 2023. In this study, 98 blood samples related to patients suspected of kidney transplant rejection referring to Labbafinezhad hospital in Tehran were collected. The samples were tested by the GeneProof Cytomegalovirus (CMV) PCR Kit to determine HCMV viral load. ROC curve analysis was used to determine the viral load cutoff point.

Findings: HCMV viremia was detected in 18 (18.36%) of 98 transplant recipients. The median viral load in the HCMV viremia group was 24914.0 IU/ml (5147.0-155106.5). The optimal cut-off value for HCMV was determined 778 IU/ml using ROC analysis. Duration of time after transplantation in the viremia and no viremia groups was 120.5 and 46 months, respectively with a statistically significant difference (P=0.014).

Conclusion: This study provides valuable insights into the prevalence of HCMV viremia and its associated risk factors in kidney transplant recipients suspected of rejection. The study also highlights the importance of post-transplant monitoring and preventive measures to address viral infections. . Long-term studies with larger sample sizes are needed to evaluate the role of factors influencing the occurrence of viremia after transplantation.

Keywords: Human cytomegalovirus, Kidney transplantation, Viral load

CITATION LINKS

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Introduction

Kidney transplantation is a life-saving treatment for people with end stage renal disease (ESRD) ^[1]. It is estimated that approximately 1.4 million people have undergone transplantation worldwide, and this process is increasing by 8% each year. In Iran, 24 transplants per million people are performed annually, meaning that around 1,700 people undergo a kidney transplant each year ^[2]. Immune-mediated graft rejection is a frequent consequence that lowers the survival of transplanted organs ^[1]. Although annual mortality from transplant rejection has now decreased to less than 5%, infectious complications continue to pose a serious threat to successful outcomes after transplantation^[3]. Among infectious pathogens in transplant recipients, viral infections are common in kidney transplant recipients and can have deleterious consequences on life expectancy and transplant outcomes ^[4]. Human cytomegalovirus (HCMV) is the most common viral infection following kidney transplantation^[5]. HCMV has the largest genome among herpes viruses and belongs to the Herpesviridae family and the Herpesvirine subfamily. This virus has a double-stranded DNA genome with 235,000 base pairs and 165 open-reading frames ^[6]. Studies have shown that the epidemiological incidence of HCMV infection and disease kidney transplantation after reaches 40-80% ^[7]. The prevalence of HCMV in Iran varies across different studies and populations. In a systematic review of the prevalence of HCMV in the Middle East and North Africa (MENA) region, HCMV IgG seroprevalence ranged from 8.7%-99.2%, and CMV incidence in these countries ranged between 1.22% and 77% in transplant and transfusion recipients^[8]. HCMV can have indirect effects caused by the effect of the virus on the host immune response,

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including acute allograft rejection, decreased long-term graft function, increased risk of other opportunistic infections, chronic and decreased allograft nephropathy, patient survival ^[9,10]. Therefore, identifying factors affecting HCMV infection after kidney transplantation is important for the prevention, management, and treatment of this disease^[7]. With current preventive strategies and the use of ganciclovir and valganciclovir, the incidence of HCMV is about 17-37% in the first 100 days after transplantation ^[11]. Quantitative detection of HCMV DNA is a valuable diagnostic tool for detecting HCMV in the early stages of

infection before disease occurs ^[12,13]. **Objectives:** The purpose of this study is to evaluate the viral load of active HCMV infection in the plasma samples of individuals suspected of kidney transplant rejection and to investigate host and risk factors associated with the activation of this virus in these patients.

Materials & Methods

Study design and sampling: In this cross-sectional study, plasma samples were collected from 98 kidney transplant patients at Labbafinezhad hospital in Tehran between December 2022 and June 2023. The criteria for inclusion of samples in the study were as follows: I. Blood samples from kidney transplant recipients who were suspected of transplant rejection based on physician's diagnosis. II. Complications after transplantation include: fever for more than a day, dysuria, edema in the hands and feet and around the eyes, sudden pain in the transplanted kidney, vomiting, and diarrhea, shortness of breath, hematuria, strangury, dizziness, cellulitis, jaundice, elevation, proteinuria, creatinine and oliguria. The occurrence of at least two of these complications was the criterion for participation in this study. III. The study group consisted of adults over 18 years old. Patients whose medical records were not complete were excluded. From each patient, 2.5 ml blood samples in EDTA anticoagulant were collected and their plasma was separated. This study was approved by the Ethics Committee of Tarbiat Modares University (IR.TUMS.SPH.REC.1395.876) and informed consent was obtained from the participants. Patients' demographic, clinical, and laboratory information were collected

from their medical records.

DNA extraction: HCMV genomes were extracted from 200 μ L plasma samples using ROJE DNall VirAll Kit QP EN (Tehran, Iran) according to the manufacturer's instructions. The extracted DNA was dissolved in 50 μ L of elution buffer and stored at -70 °C for further analysis.

HCMV viremia assay by quantitative real time PCR (qPCR): HCMV viral load was tested using GeneProof Cytomegalovirus (CMV) PCR Kit (based in the Czech Republic.) based on the amplification of the 4 IE antigen. Quantitative real-time PCR (qPCR) was carried out using StepOnePlusTM Real-time PCR System (Applied Biosystems International, Inc, Switzerland). The Diagnostic specificity and sensitivity of the kit are 90.67% and 92.86%, respectively. The Detection limit is 122.594 IU/ml. An internal standard (IS) in the kit was used to control the quality of extracted DNA and to control the possible inhibition of the PCR reaction. The mixture of primer, probe, and MasterMix is designed in one vial. The kit contains 4 calibrators with concentrations of 10^1 , 10^2 , 10^3 , and 10^4 IU/µL. First, we mixed 1 µL of IS with 10 μ L of the extraction products. Samples were assessed in a 40 µL reaction mixture consisting of 30 µL of MasterMix and 10 μ L of extraction products mixed with IS. We added 10 µl of each positive control, negative control, and 4 calibrators into the individual PCR tubes. The Real-time

PCR thermal conditions were as follows: 2 min at 37 °C (hold), 10 min at 95°C (initial denaturation), followed by 45 cycles of 5 s at 95°C, 40 s at 60°C, and 20 s at 72°C.

Statistical analysis: Statistical analysis was performed using SPSS 26 software, and ROC curve analysis was used to determine the viral load cutoff point. Qualitative variables are described using frequency and percentage. The normality of quantitative variables was evaluated using the Kolmogorov-Smirnov test. Continuous normal variables were reported based on the mean and standard deviation (Mean±SD) and non-normal quantitative variables were reported using the median and interquartile range (Median [IQR]). The association between qualitative variables was determined using chi-square or Fisher's exact test. The comparison of the mean of the quantitative variables between the two groups was done using the t-test or Mann-Whitney test. The p-value less than 0.05 was considered significant.

Findings

Demographic and clinical characteristics: The mean age of the subjects was 50.7 ± 16.7 years. 57 patients (58.2%) were male and 41 (41.8%) were female. The median interval between transplantation and sampling was 66 months (7-149). The most underlying diseases in the studied population included hypertension (71.4%), diabetes mellitus (23.5%), polycystic kidney (23.5%), and glomerulonephritis (20%). The most immunosuppressive drugs were prednisolone acetate (94%), mycophenolate mofetil (80%), and tacrolimus (53%). In the analysis of clinical symptoms in subjects suspected transplant rejection fever and chill (38.7%), dysuria (26.5%), elevated creatinine (25.5%), renal pain (19.3%), vomiting (14.2%), organ edema (13.2%), shortness of breath (9.2%), strangury (8.1%), dizziness (6.1%), oliguria (6.1%), diarrhea (5.1%), proteinuria (5.1%), cellulitis (3%), hematuria (2.0%), jaundice (1.0%), and other symptoms (10.2%) were the most common. There were no differences in baseline and biochemical characteristics between the viremia and no viremia groups including age (P=0.59), gender (P=0.43), underlying diseases (P>0.05), number of transplants (P=0.83), donor type (living P=0.85, cadaver P=0.85), serostatus of HCMV donor and transplant recipient (P=0.88), drug regimen (P>0.05), elevated creatinine (P=0.32), and decreased GFR (P=0.84). Only the "time after transplantation" has a statistically significant difference between the two groups (in groups with viremia and groups without viremia is 120.5 and 46 months, respectively (P=0.014) (Table 1).

Table 1) Demographic and clinical characteristics of human cytomegalovirus (HCMV) in viremia group vs. theno viremia group of kindney tranplant recipients

Demographic	No Viremia n=80	Viremia n=18	<i>P</i> -value			
Age	49.417.35±	52.6 ±12.8	0.110			
Sex (Male)	48(60.0)	9(50.0)	0.437			
Sex (Female)	39(40.0)	9(50.0)				
Duration post transplantation (month)	46[3.1-142.8]	120.5[39.3-227.3]	0.014*			
Total number of transplant						
1	61(76.3)	15(83.3)	_			
2	17(21.3)	3(16.7)				
3	2(2.5)	0(0.0)	0.838			
Donor type						
Live donor	56(70.0)	13(72.2)	0.852			
Cadaveric	24(30.0)	5(27.8)	0.852			
CMV Antibody status pre transplantation						
D+R+	70(87.5)	15(83.3)	_			
D+R-	4(5.0)	2(11.1)	_			
D-R+	4(5.0)	1(5.6)	0.885			
D-R-	2(2.5)	0(0.0)				
Immunosuppression regimen & Antimetabolite						
Mycophenolate mofetil/Myfortic	65(81.3)	15(83.3)	1.00			
Cyclosporine	37(46.3)	12(66.7)	0.191			
Tacrolimus	47(58.8)	6(33.3)	0.069			
Azathioprine	4(5.0)	2(11.1)	0.589			
Anti thymocyte globulin	37(46.3)	4(22.2)	0.062			
Prednisolone Acetate	76(95.0)	18(100.0)	0.591			
Anti-viral drugs	5(6.3)	15(83.3)	< 0.001*			
Biochemical parameters						
Creatinin increase rate	0.8[0.3-1.87]	1.45[0.38-2.4]	0.321			
GFR reduction rate	-24.1±20.4	-25.2±16.4	0.840			

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Determination of HCMV DNA cutoff value for qPCR: Using ROC curves, optimal cutoff point for viral load by qPCR in plasma was calculated. In this study, the optimal cut-off point for HCMV was determined 778 IU/ml using ROC analysis (Figure 1 & Table 2).

Of the 98 patients, HCMV viremia was detected in 18 (18.36%) transplant recipients. The median viral load in the HCMV viremia group was 24914.0 IU/ ml (5147.0-155106.5). Herein, all kidney transplant recipients receive post-transplant prophylaxis including Valganciclovir (Valcyte), according to their GFR. In this study, more than half of patients with suspected kidney transplant rejection who developed



Figure 1)Receiver operating characteristic (ROC) curve for human cytomegalovirus (HCMV) viral load in kidney transplant recipients

Table2) Area under the curve and receiver operating characteristic (ROC) test result for human cytomegalovirus (HCMV) viral load in kidney transplant recipients

Area SE	SE	<i>P-</i> Value _	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.776	.088	0.082	0.604	0.948

HCMV viremia received antiviral therapy (83.3%) in addition to immunosuppression. The odds ratio (OR) of HCMV viremia in the group that used tacrolimus was 2.84 times higher than the OR of viremia in the group that did not use tacrolimus (OR:2.84, 95% C.I:0.97-8.35, P=0.057) and it was significant at the 10% level.

The OR of HCMV viremia in the group that used anti-thymocyte globulin (ATG) was 3.01 times higher than the OR of viremia in the group that did not use ATG (OR:3.01,95% C.I:0.91-9.94, *P*=0.071) (Table 3).

Discussion

Viruses are among the most common opportunistic infectious agents during and after transplantation. Therefore, preventive measures such as pre-transplant screening, preventive antiviral therapy, and posttransplant laboratory diagnosis and virus detection are important ^[14]. This study was conducted with the aim of determining HCMV viral load in the plasma of kidney transplant recipients using the qPCR method. HCMV molecular assays are useful due to high sensitivity in measuring viral load ^[14,15]. HCMV viremia was detected in 18 (18.36%) recipients, and among the host factors only "Time after transplant" has a statistically significant difference in the viremia and no viremia groups (Table 1). In line with our study, Afshari A et al in Shiraz, performed ROC curve analysis to understand the sensitivity and specificity of HCMV viremia to distinguish between groups infected with latent and active viruses and evaluated the expression level of CMV miRNAs in active vs. latent CMV infected KTRs ^[10]. A study conducted by Pullerits K et al in 2022 to assess the incidence and predictors of posttransplant infections in kidney transplant recipients showed that out of 962 recipients, HCMV, Epstein-Barr virus (EBV), BK virus (BKV) and JC virus (JCV) were detected

	Odds ratio	95% C.I for Odds ratio		D-valuo
	Ouusiatio	Lower limit	Upper limit	<i>I</i> -value
Tacrolimus	2.848	0.971	8.357	0.057
Anti-thymocyte globulin	3.012	0.912	9.949	0.071

 Table 3) The odds ratio of human cytomegalovirus (HCMV) infections in kidney transplant recipients used tacrolimus or anti-thymocyte globulin

in 13.8%, 11.3%, 8.9% and 4.4% of recipients. The median time to viremia after transplantation was 9 months for HCMV and 45 months for EBV. BKV and JCV infection occurred at a median of 13 and 15 months after transplantation, respectively. Factors associated with an increased risk of infection with these viruses after transplantation were female recipient gender, higher number of HLA mismatches, lower baseline glomerular filtration rate (eGFR), HCMV seropositive donor ^[16]. But in our study, no statistically significant differences was observed in this variables in viremia and no viremia groups (P>0.005) (Table 1). Rahbar M et al (2019) conducted a study on simultaneous detection of opportunistic viral infections in kidney transplant patients in Tehran, Iran. Plasma and urine samples were collected from 101 transplant patients with elevated creatinine, and 15 (14.8%) tested positive for HCMV, 10 (9.9%) for EBV, and 19 (18.8%) for BKV. The mean HCMV levels in the patients' blood and urine were 3.7×10^5 and 3.3×10^5 IU/ml, respectively ^[17]. In our study, HCMV viremia was detected in 18.36% of transplant recipients, which is close to the level of viremia reported in this study. In another study conducted in Sweden in 2010 by Sund F et al, HCMV viral load was evaluated by PCR in kidney transplant recipients, all of whom had HCMV D+/R+ serostatus. 16 (94%) of the 17 patients were positive for HCMV DNA in plasma at least once. The median HCMV DNAemia level in

patients without prophylactic treatment was 8400 (2.9-820 million) copies/ml, and no association between CMV viral load and graft function was found one to three years after transplantation ^[18]. In our study, the median HCMV viral load in kidney transplant recipients was 24914.0 IU/ml (5147.0-155106.5), regardless of the patients' serostatus. In a 2016 study conducted in Egypt by Tarek G et al, the distribution of the main causes of ESRD was as follows: hypertension 31.8%, diabetes 15.5%, urinary tract infection 8.8%, kidney stones 8.4%, unknown factors 17.7%, primary glomerulonephritis 3.7%. and, drug 3.5%^[19]. In our study, hypertension was the main cause of ESRD with a prevalence of 71.4%, followed by diabetes mellitus and polycystic kidney both with a prevalence of 23.5%.

In 2022, Pullerits et al reported that treatment with cyclosporine (instead of tacrolimus) and a greater number of immunosuppressive drugs are risk factors for viremia ^[16], while in our study no significant association was found between the use of immunosuppressant and the development of viremia (P>0.005). However, the OR of HCMV viremia in the group that used the tacrolimus drug was 2.84 times the OR of viremia in the group that did not use tacrolimus (OR:2.84, 95% C.I:0.97-8.35, P=0.057) and it was significant at the 10% level. In addition, the probability of HCMV viremia in the group that used ATG was 3.01

times higher than the probability of viremia in the group that did not use ATG (OR:3.01, 95% C.I:0.91-9.94, P=0.071) (Table-3). It should be noted that the prevalence of viral infections in kidney transplant patients varies from country to country and many

varies from country to country and many factors play a role in determining the risk of infection, such as immunosuppression status, postoperative care, etc. Socio-economic conditions and low health standards also contribute to the increase in infectious complications in developing countries ^[20]. Considering the studies carried out in this field and the use of sensitive PCR methods in these studies, the differences observed in distinct studies may be due to the different number of samples, study duration, and the heterogeneity of the population studied, the interval between transplantation and the clinical manifestations.

The number of patients in this study was relatively small and the follow-up period was limited. Long-term studies with larger sample sizes are needed to evaluate the role of factors influencing the occurrence of viremia after transplantation.

Conclusion

Rapid and timely diagnosis of viral activation in kidney transplant patients are effective for the patient management and use of appropriate preventive and therapeutic strategies. HCMV molecular assays are useful due to high sensitivity in measuring viral load and rapid diagnosis of infection, although the lack of standardization and the absence of a specified cut-off among molecular assays make it difficult to quantify CMV DNA. Plasma samples from kidney transplant recipients were from all phases (early, middle, and late) after transplantation. Therefore, investigation of viremia is recommended in kidney transplant recipients in whom the infection occurred within 1 year of transplantation.

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Ethical Permission: This study was approved by the Ethics Committee of Tarbiat Modares University (IR.TUMS.SPH. REC.1395.876).

Authors Contribution: SSM designed and supervised the work. MEK collected the samples and performed the laboratory tests. SSM wrote the manuscript. MRJ contributed in sample collection.

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