Cytomegalovirus Viremia and Risk Factors in Renal Transplant Recipients in Iran: a Prospective Case-control Study

Najmeh Parhizgari
Tehran University of Medical Sciences
Farhad Rezaei
Tehran University of Medical Sciences
Mohamad-Reza Khatami
Tehran University of Medical Sciences
Sayed Mahdi Marashi
Tehran University of Medical Sciences
Mohammad Farahmand
Tehran University of Medical Sciences
farzaneh Behnezhad
Tehran University of Medical Sciences
Mohadeseh Zarei-ghobadi
Tehran University of Medical Sciences
Monireh Derakhshani
Tehran University of Medical Sciences
Fatemeh Ajami-Nezhad
Tehran University of Medical Sciences
Talat Mokhtari-Azad (mokhtari@hotmail.com)
Tehran University of Medical Sciences

Research

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Abstract

Background: In spite of effective anti-viral drugs and risk-balanced prophylaxis regimen, cytomegalovirus (CMV) remains a major reason of morbidity in kidney transplant patients. The aim of present study was to evaluate CMV viral load and laboratory findings correlation with CMV viremia graft origin and investigation on late or early onset CMV infection in kidney transplant recipients with CMV viremia.

Methods: This research designed as a prospective case-control study based on CMV PCR test and exclusion of other viral infection among renal transplant patients in Iran.

Results: From 192 examined patients, 153 participants were qualified to enter the study: 43 in case (with CMV viremia) and 110 in control group (CMV negative test). Statistical analysis performed to identify the risk factors raising this viral viremia among kidney transplant patients.

Conclusion: Receiving a renal graft from a deceased donor significantly raise the chance of viremia in renal transplant patients. The median month of CMV viremia occurrence was month 4 after transplantation in both groups. Serum laboratory testing showed creatinine and platelets significantly raised and reduced, retrospectively in the case compare to control group. Our results indicating the viremia has not affected the survival of the allograft or patient.

Background

In spite of effective anti-viral drugs and risk-balanced prophylaxis regimen, cytomegalovirus (CMV) remains a major reason of morbidity in kidney transplant patients (1). In the renal transplant (RT) patients, infection occurs primarily or as reactivation of latent virus depends on kidney donor or recipient CMV serostatus (2). CMV reactivation can result in CMV disease or direct effects of the disease related to the presence of high rates of viral replication and lytic virus production (3), and indirect effects caused by virus interaction with the most immune response acute rejection, graft dysfunction, opportunistic infections, diabetes mellitus and malignancies the indirect effects of CMV are assumed to be mediated by cytokine and chemokine production (4, 5).

Re-emergence of CMV viremia could occur as a ‘early onset’ or ‘late onset’ infection (6). Ten to 50% of allograft recipients develop early onset CMV infection after or during receiving anti-viral prophylaxis. Approximately half of these patients will develop clinical manifestations of disease, and up to 30% of successfully treated cases of CMV disease will recur. CMV infection characteristically presents during the first 6 months after organ transplantation, and recurrences occur within 3 months of completion of therapy for the initial episode. Late CMV disease is defined as that which presents 16 months after organ transplantation, and it is most often related to the need to increase the level of immunosuppression because of late episodes of rejection (7, 8).

Quantitation of CMV in RT patients allows for assessment of the degree of CMV replication, which is expressed as the absolute viral load value. Trends in viral loads over time (viral load kinetics) directly

correlate with the likelihood of severe CMV disease (9). In a series of prospective studies, peak CMV load during active infection is identified as a major risk factor that correlates with the development of CMV disease (9).

There are reports in RT patients indicating the allograft origin might change the risk of CMV infection after transplantation. The studies show that outcome of living donor kidney transplantation has been better than that of deceased donor kidney transplantation (10) Deceased donors, particularly donors whose organs are accepted based on expanded criteria, might contribute to an increase in the infection risk through intensive immunosuppression or donor-derived nosocomial organisms (11). Recently, in a cohort study in the Europe, a deceased donor transplantation was identified to be associated with increased incidences of CMV viremia (12).

In Iran, a comprehensive study on CMV viral load and laboratory findings correlation with CMV viremia, graft origin and investigation on late or early onset CMV infection in kidney transplant recipients with CMV viremia have not been performed. We therefore conducted a prospective case-control analysis to examine the above factors which could be affected by the virus viremia or have a correlation to increase the risk of developing the infection among renal transplant patients in Imam Khomeini hospital, one of the main center of kidney transplantation in Iran.

Methods

Study design and setting

The study design was prospective case-control, the recruited subjects comprised 153 RT patients referred to Imam Khomeini Hospital, Tehran, Iran between February 2019 and February 2021. To select the case group, the suspected or randomly found patients to CMV infection were sampled and the control group were collected from RT patients with no typical symptoms of CMV infection. from 192 patients who were examined and tested, 43 and 110 patients were recruited in the case and control group, respectively (Supplementary 1,2). The inclusion criteria included the adult patients (age ≥ 18 years) including outpatients and hospitalized RT patients who received a live or deceased donor kidney transplant in Imam Khomeini Hospital. All RT patients in both groups were donor positive and recipient positive, either symptomatic (fever, low glomerular filtration rate and signs of urinary tract infection) or asymptomatic. A viral load of 200 copy/ml and above defined as viremia to enter to the case patients. BKV, HHV6 and EBV PCR performed on all the plasma samples of two groups and the negative results provide the recruitment to enter the case or control categories (Figure1).

The study was evaluated and approved by the Ethical Committee of Tehran University of Medical Sciences. Before specimen collection, informed consent was obtained from all patients. The subjects' general data (age, gender, months after transplantation, donor source, and date of transplantation) and underlying diseases caused kidney loss of function recorded in a questionnaire. From each participant 3 ml aliquot of whole blood sample was withdrawn, and plasma was separated immediately from 1 ml using EDTA as anti-coagulant and were stored at -70˚C in RNase DNase free micro-tubes for viral nucleic

acid extraction. Two ml of blood sent to the diagnostic laboratory for laboratory parameters including white blood cell count, platelet count, serum FBS, serum creatinine, serum uric acid, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and Alkaline phosphatase (ALP) (Figure1).

Immunosuppressive Therapy

In IKH, most patients received routine triple immunosuppression with a calcineurin inhibitor (CyA/tacrolimus), mycophenolate sodium or mycophenolate mofetil and oral prednisolone. Those patients at risk received thymoglobulin induction. Target tacrolimus trough levels are typically between 5 and 10 µg/L for the first 3 months, with the dose of prednisolone reduced to 5–10 mg daily by 3 months. Biopsy-proven T cell mediated rejection episodes were managed with intravenous methylprednisolone, with T cell depleting antibody being prescribed for steroid- resistant rejection episodes. CMV prophylaxis with valganciclovir was administered for 100 days to patients.

DNA extraction and PCR for CMV, BKV, HHV6 and EBV

Viral nucleic acids were extracted from 200ul of plasma samples using Roche viral nucleic acid extraction kit (Roche, Basel, Switzerland), according to the manufacturer's instruction (Supplementary1). BKV, HHV6 and EBV infection have examined by conventional PCR as described before (13, 14). (Supplementary2)

Statistical analysis

Descriptive statistics including means and standard deviations of quantitative variables and frequencies (%) of qualitative variables were computed. Continuous and categorical variables were presented as median (IQR) and n (%), respectively. To compare differences between different groups we used the Wilcoxon rank-sum test, $\chi 2$ test, or Fisher's exact test where appropriate. To explore the risk factors associated with CMV viremia and allograft survival or rejection. Following analysis based on CMV PCR test, multivariate logistic regression models were used to control possible cofounders. A two-sided α of less than 0·05 was regarded statistically significant. Statistical analyses were done using R version 4.0.3 (2020-10-10).

Results

From among all the 192 surveyed patients, based on the qPCR test and CMV viral load, 43 patients had more than 200 copy of CMV genome in plasma. The study population included 43 patients and 110 controls. Univariate analysis revealed that CMV positive real time PCR test was significantly associated with having a lower platelets and higher blood creatinine (Supplementary 1). CMV infection has occurred significantly ($P < 0.05$) more in patients who received the kidney from a deceased donor. However, this univariate analysis did not show any significant differences between patients and controls regarding the

months after transplantation between two groups (Table 1,2). The main reason of the patient's loss of function was blood pressure (72%) followed by diabetes (36 %) (Figure 2).

Demographic Profile of Transplant Recipients

The descriptive variables categorized by CMV molecular test results are presented in table 1. Among the case-control participants, mean age was 51.3 years. The majority of patients were males (71%), and primary renal disease was chronic glomerulonephritis in 57% of CMV positive patients. About 27% of the kidney transplant recipients were from living related donors.

Risk Factors of CMV viremia

According to multivariate logistic regression models, age, WBC, platelets, FBS, creatinine, uric acid and donor source had P-values of less than 0.2 and entered to multivariable analysis. The result of models indicates that receiving organ from a deceased donor significantly augments the risk of CMV infection in the case group (Table 3).

Discussion

While CMV serostatus play an important role in CMV infection development in kidney Transplant recipients, it is yet not clear whether there are other risk factors could act as well. The present study aimed to investigate the risk factors which increase CMV infection occurrence in this group.

In the present study, we compare the results of serum laboratory tests in CMV positive patients to the negative patients to find the tests which significantly differ between two groups. Besides, we investigate the incidence of early or late CMV infection, risk factors and consequences of CMV viremia among kidney transplant recipients compare to the control patients.

The presented results indicate that RT patients who received the allograft from deceased donor faced with higher risk of CMV viremia after transplantation mostly among the first four months. Recent analysis among RT patients suggests grafting from a cadaveric would rise the risk of CMV viremia.

CMV viremia in our study emerged mostly after 4 months after transplantation, likewise, in a cohort study in the Europe, among 19.2% of CMV + patients, viremia emerged within the first three months after transplantation(12). given acute rejection episodes occurred within the first month post transplantation, the association with CMV infection in this period may be attributed to excessive immunosuppression associated with acute rejection treatment. Infection recuurence probably promotes by the initial immunosuppression and cessation of anti-viral prophylaxis. Notably, it is suggested that during this time, patients be monitored and treated by antiviral prophylaxis in accordance with the higher risk of CMV infection. Even though, there was no significant different between the time of CMV detection among both CMV+/- groups, our results presenting that early CMV infection emerges in 4 months after transplantation that may consider an alarming time to examine the presence of CMV viremia in the RT patients.

The results show that late onset CMV infection has not occurred frequently since the late months among the investigated patients recorded on month 7 of receiving the kidney. In other studies, late-onset CMV disease still develops in approximately 18% of patients even in the presence of either prophylactic strategies (15, 16).

In the present study, CMV viremia did not significantly affect the allograft and patient's survival contrary to other studies (17). Given we investigated seropositive RT patients and in patients who are CMV seropositive, viral replication occurs in the context of pre-existing immunity, hence the observed replication rate is slower in such individuals. As a result of the widespread use of antiviral prophylaxis and preemptive therapy, the incidence and severity of CMV disease and its indirect effects are significantly reduced. The incidence of CMV in the renal transplant population is estimated to be between 8 and 32 percent (7).

Patients in the case group displayed worse serum creatinine values post transplantation, albeit without significant differences in graft and patient survivals (5). Serum Creatinine raised and platelets diminished. This result confirms the precedent facts which CMV infection should be considered in any renal transplant recipient who has a rise in creatinine even if symptom-free (18).

Our results did not investigate the local detection of CMV in the allograft, it is important to consider that the mere detection of CMV does not essentially exclude the presence of CMV in the blood. Indeed, lack of serum CMV positive test does not completely rule out CMV infection in these patients. Since transient periods of CMV viremia had been found in some cases due to the compartmentalized or localized CMV diseases (19, 20). In the present study, all subjects had received CMV antivirals including ganciclovir (1.25 mg/kg IV daily as induction for 1 month, which then was switched to oral valgancyclovir) or valcyte (450 mg, according to their plasma creatinine levels) for the first 3 months post transplantation. In the cases of CMV DNAemia, some patients did not show any typical syndromes of CMV infection.

In our investigation, we selected CMV viremia detection by finding the virus DNA in the patients' blood. Given new diagnostic method based on the amplification of CMV RNA in blood samples has been commercialized, we recommend that future studies categories the case-control patients by these novel techniques (21, 22)

Conclusions

In conclusion, receiving a kidney from deceased donor significantly increased incidence of CMV viremia in studied RT patients. Elevated level of creatinine and reduction of platelets in serum strongly changed during the infection which suggest considering asymptomatic CMV infection among RT patients with high serum creatinine. No late CMV infection detected in our case group and the viremia has not affected the survival of the allograft or patient. Future case-control studied are recommended to perform based on CMV diagnosis by viral late gene expression.

Abbreviations

CMV: Cytomegalovirus, BKV: BK polyomavirus, HHV6: Human herpesvirus-6, EBV: Epstein Bar Virus, PCR: Polymerase chain reaction, RT: Renal Transplant, EDTA: Ethylenediaminetetraacetic acid, SGOT: serum glutamic oxalacetic transaminase, SGPT: serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase, WBC: White Blood Cell, FBS: Fasting Blood Sugar, DNA: Deoxyribonucleic Acid, RNA: Ribonucleic Acid, IQR: interquartile range.

Declarations

Ethics approval and consent to participate

The study was evaluated and approved by the Ethical Committee of Tehran University of Medical Sciences. Before specimen collection, informed consent was obtained from all patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no competing interest to declare. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

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Contribution

Conceived and designed the experiments: NP, FR, TMA. Performed experiments: NP, FB. Contributed materials/analysis: NP, MRK, SMM, MD, MZG, FAN. Analyzed the data: NP, MF. Wrote the paper: NP, FR, TMA.

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Tables

Table 1: Laboratory findings and risk factors in renal transplant patients case-control based on CMV Real Time PCR test

Characteristic	N	Overall (N = 153)	Negative (N = 110)	Positive (N = 43)	p-value2
Sex	153				0.8
Female		44 (29%)	31 (28%)	13 (30%)	
Male		109 (71%)	79 (72%)	30 (70%)	
Age	153	54 (39, 61)	55 (41, 62)	45 (39, 60)	0.2
WBC	151	7.60 (5.70, 9.30)	8.00 (5.90, 9.50)	6.70 (5.43, 8.75)	0.10
Platelets	151	197 (164, 253)	202 (172, 264)	182 (153, 221)	0.019
FBS	145	105 (89, 130)	108 (89, 134)	104 (84, 120)	0.2
Creatinine	153	1.41 (1.12, 1.79)	1.33 (1.11, 1.73)	1.57 (1.31, 2.14)	0.008
Uric. Acid	129	5.80 (5.00, 6.70)	5.70 (4.93, 6.70)	6.00 (5.60, 6.80)	0.2
SGOT	90	20 (15, 25)	21 (16, 25)	20 (14, 26)	0.4
SGPT	90	32 (19, 50)	34 (19, 50)	30 (20, 43)	>0.9
ALP	80	232 (178, 330)	243 (183, 352)	229 (178, 308)	0.7
Months after transplantation	153	5 (2, 15)	5 (2, 22)	4 (3, 7)	0.4
Survival	153				>0.9
Rejected		2 (1.3%)	2 (1.8%)	0 (0%)	
Survived		151 (99%)	108 (98%)	43 (100%)	
Donor sources	153				0.002
Deceased donor		112 (73%)	73 (66%)	39 (91%)	
Living donor		41 (27%)	37 (34%)	4 (9.3%)	

[1]n (%); Median (IQR)

[2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

* months after transplantation

Table 2: Laboratory findings and risk factors in renal transplant patients case-control groups based on Rejected and Survived allograft

Characteristics	Overall (N)	Rejected (N = 153)[1]	Survived (N = 2)[1]	p-value[2]
Age	153 54 (39, 61)	50 (42, 57)	54 (39, 61)	>0.9
WBC	151 7.60(5.70,9.30)	9.50 (9.30, 9.70)	7.60 (5.70, 9.30)	0.2
Platelets	151 197 (164, 253)	135 (125, 145)	197 (166, 253)	0.070
FBS	145 105 (89, 130)	102 (99, 106)	105 (88, 130)	0.8
Creatinine	153 1.41 (1.12, 1.79)	1.10 (1.08, 1.13)	1.41 (1.12, 1.79)	0.2
Uric Acid	129 5.80 (5.00, 6.70)	NA (NA, NA)	5.80 (5.00, 6.70)	
SGOT	90 20 (15, 25)	21 (20, 22)	20 (15, 25)	0.9
SGPT	90 32 (19, 50)	46 (43, 49)	32 (19, 48)	0.3
ALP	80 232 (178, 330)	NA (NA, NA)	232 (178, 330)	
Months after transplantation	153 5 (2, 15)	8 (4, 12)	5 (2, 16)	0.6
Donor sources	153			0.5
Deceased donor	112 (73%)	1 (50%)	111 (74%)	
Living donor	41 (27%)	1 (50%)	40 (26%)	
CMV test	153			>0.9
Negative	110 (72%)	2 (100%)	108 (72%)	
Positive	43 (28%)	0 (0%)	43 (28%)	

[1] Statistics presented: Median (IQR); n (%)
[2] Statistical tests performed: Wilcoxon rank-sum test; Fisher's exact test

Table 3: Final variables correlate with CMV infection in RT patients

Characteristic	OR[1]	95% CI[1]	p-value
age	0.98	0.95, 1.02	0.3
WBC	0.92	0.75, 1.13	0.5
Platelets	0.99	0.98, 1.00	0.006
FBS	1.00	0.98, 1.01	0.4
Creatinine	0.69	0.25, 1.87	0.5
Uric Acid	1.06	0.76, 1.48	0.7
Donor sources			
Deceased donor	—	—	
Living donor	0.29	0.07, 0.96	0.057

[1]OR = Odds Ratio, CI = Confidence Interval

Figures

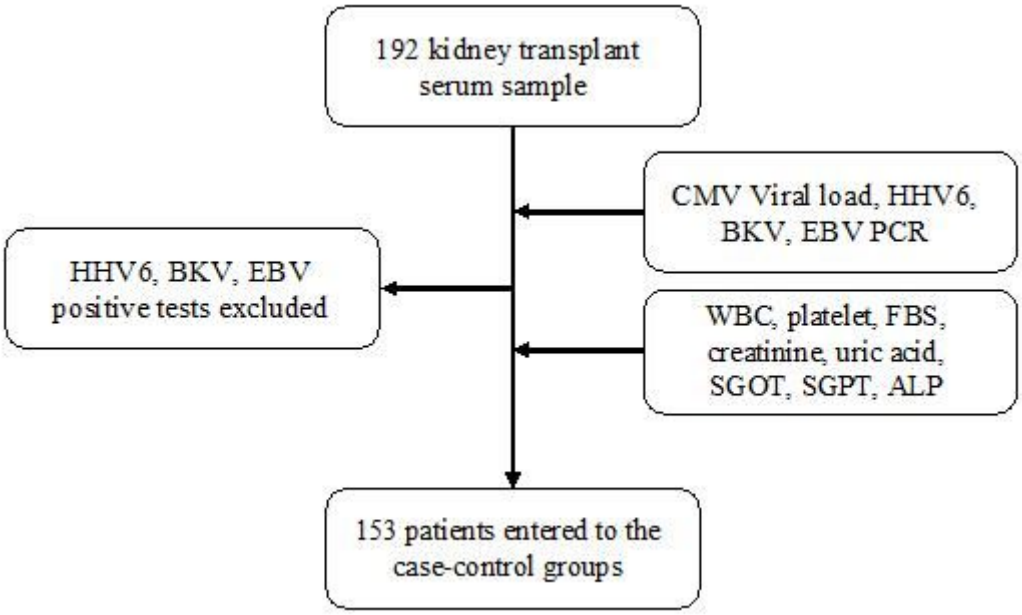

Figure 1

Flowchart of the prospective case-control study design. Renal transplant patients with merely CMV infection included in the case and patients with none of CMV, EBV, HHV6 and BKV infection with the laboratory test results entered the control group.

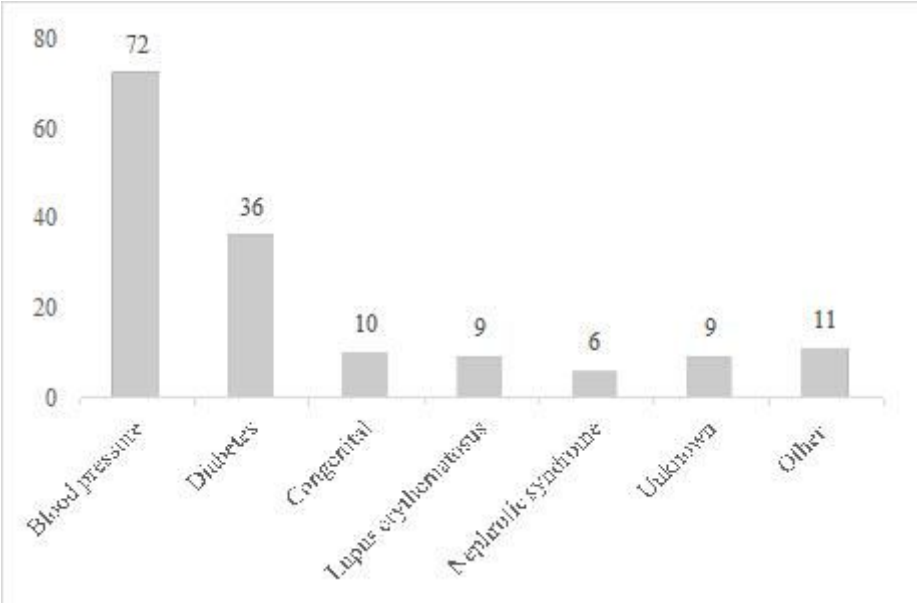

Figure 2

The frequency of kidney loss of function in the investigated patients in both case-control patients.

Supplementary Files

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