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Research Article

DETECTION OF HUMAN CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANT PATIENTS BY REAL TIME POLYMERASE CHAIN REACTION (PCR) TECHNIQUE, ISFAHAN, IRAN

Abbasali Pourazar and *Mehrnaz Shanehsazzadeh
Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Hezarjerib Ave., Isfahan, Iran
**Author for Correspondence*

ABSTRACT
Cytomegalovirus (CMV), being common in kidney graft patients, has been investigated as main cause of mortality and morbidity. This study was carried out to detect human cytomegalovirus (HCMV) DNA in renal transplant patients in Isfahan, Iran through Real-time Polymerase Chain Reaction (PCR) testing. A total of 250 plasma samples were collected randomly from renal transplant patients at 1 month post transplantation from 2008 to 2011. CMV DNA was detected using real-time PCR. Among 250 renal transplant patients, 180 were males and 70 were females. The results revealed that HCMV DNA was present in 185 patients' plasma samples (74%) using Real-time PCR. HCMV detection by real-time PCR in the present study indicated a high prevalence among renal transplant patients in Isfahan. Further study should be considered to determine the prevalence of HCMV disease at the national level.

***Key Words*:** *Cytomegalovirus, CMV DNA, Real-Time Polymerase Chain Reaction (PCR), Kidney Graft Patients, Iran*

INTRODUCTION
Human cytomegalovirus (HCMV) is belongs to the family Herpesviridae (Mocarski and Courcelle, 2001). HCMV is strictly species specific, as are the other cytomegaloviruses with persistence in the host in a latent state after primary infection (Emery, 2001). It is a ubiquitous virus with the seroprevalence as 30-00% in different countries. Transmission of HCMV can occur via direct contact with infectious body fluids, Blood, and blood products or transplanted organs (Emery, 2001). This virus Causes a significant health threat to immunocompromised individuals, and is an important factor of morbidity and mortality, especially in transplant patients (Benz et al., 2002). When the host immune system is compromised, the virus insertion occurs to host cells (Emery, 2001). Among risk factors known for the development of HCMV disease, viral load is a major indicator for development of HCMV disease (Emery, 2001).
One way for diagnosing HCMV disease is based on clinical symptoms, but HCMV can be mistaken with Epstein-Barr virus (EBV). The serological and molecular techniques are common for confirmation of disease ((Kubar et al., 2005). Some information is documented about HCMV or related disease prevalence in Iran (Lashini et al., 2011) but there is no sufficient information for Isfahan city with an health-related focus in the state. Therefore, the aim of the present work was to find the prevalence of HCMV infection in renal transplant patients via real-time polymerase chain reaction (PCR).

MATERIALS AND METHODS
Patients and samples
Renal transplant patients (N=250) with no antiviral therapy were recruited into this study from Isfahan Hospitals, Iran from 2008 to 2011. All patients in this study suffered from symptoms like fever, diarrhea, hepatitis, neutropenia and/or thrombocytopenia. All patients presented 1 month after kidney transplantation. Case status and clinical information was collected by reviewing medical records including age, gender, date of transplantation, date of sample collection, and patient location during

sample collection. A total of 250 blood samples were collected from the patients. Plasma was separated by centrifugation and stored frozen at -20°C until further analyses.

CMV Real-Time PCR analyses (Yoshida et al., 2001)

Commercial ELISA kits (Biokit, Barcelona, Spain) were used to detect HCMV DNA according to the procedure described by the manufacturer. For CMV testing, DNA was extracted from 200 µL of plasma using the QIAamp DNA Extraction kit (QIAGEN, Germantown, MD, USA). For each plasma sample, 5.5 µL (1 mg/mL) Carrier RNA and 10 µL CMV internal control were added to 200 µL of AL lysis buffer following the manufacturer's recommendations. The sample was eluted in 50 µL and 20 µL were used for the assay. CMV viral load testing was carried out using the artus CMV PCR assay according to the manufacturer's instructions (QIAGEN). The artus CMV™ Master Mix contains reagents and enzymes for the specific amplification of a 105-bp region of the major immediate early antigen. A standard curve was obtained from the quantitation standard (QS) CMV DNA positive controls (CMV TM QS 1-4) provided by the manufacturer. For the PCR amplification, 20 µL of DNA sample eluate was added to 30 µL of the working master mix. The amplicons were then detected by measuring fluorescence, using the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Carlsbad CA, USA) with the following amplification conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 55°C for 1 min. At the end of the run, the data were analyzed using the SDS detection software v2.2.2 (ABI). The linear range of the CMV DNA test was from 2.3 to 5.7 log10 copies/mL. Quantitative results were reported if >2.3 log10 copies/mL (200 copies/mL); positive results below 2.3 log10 copies/mL were reported as <200 copies/mL, CMV DNA detected.

RESULTS

In the present study, there were 250 renal grafted patients aged 16-83 years old. Of these patients, 180 (72%) were male and 70 (28%) were female. HCMV DNA was detected via real-time PCR in 185/250 (74%) of the plasma samples, with viral loads ranging from <200 to 42932 copies/mL.

DISCUSSION

HCMV has remained a significant factor of morbidity and mortality in immunocompromised patients such as renal transplant recipients (Paya, 2001). Effective antiviral therapy depends on detecting HCMV disease at an early stage of infection (Yoshida et al., 2001) via a rapid and accurate procedure. Moreover, the quantification of the systemic HCMV load may provide highly sensitive and specific criteria to predict which patients will develop HCMV disease.

The present study has focused on the molecular diagnosis of HCMV in renal transplant patients in Isfahan, Iran. The 72% prevalence of HCMV DNA was found in this study that indicates a high rate of past infection, and demonstrates that HCMV infection is common in renal transplant patients in Iran. This high prevalence of HCMV may be attributed to the immunosuppressive treatment and poor health conditions (Jawetz et al., 2004). These findings might be considered alarming indicators of disease in such patients.

The prevalence of HCMV infection in the general population of African countries as well as in transplant patients is reportedly high. The seroprevalence has been reported to be 90% in Eritrea (Ghebrekidan et al., 1999), 77.6% in Ghana (Adjei et al., 2008), and 36.2% in Egypt (Agha et al., 1989). A Real-Time PCR method was used in the present study as a highly sensitive and specific method to predict which patients will develop HCMV disease. HCMV DNA by real-time PCR was detected in 185/250 (74%) patients. This might be attributed to the fact that quantification of HCMV DNA is considered both more sensitive and more specific (Zipeto et al., 1993).

There was considerable variation in the viral loads in the current study that is a function of the sensitivity and specificity of real-time PCR for detection of HCMV DNA as compared to serologic tests. A high viral load is closely correlated with their clinical status suggestive of active HCMV infection. Our results

are in agreement with that of Emery et al., (2000) who reported that HCMV load in the initial phase of active infection as well as the rate of increase in viral load both correlate with HCMV disease in transplant recipients.

In conclusion, this study was a baseline data analysis for future studies in Isfahan with a long term goal of antiviral therapy against HCMV in the Iran. Therefore, further research work should be carried out to study the epidemiology of HCMV at the molecular level in renal graft patients.

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