Short Communication

Real-time PCR and pp65-antigen test for monitoring human cytomegalovirus infection in kidney transplant patients

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Received: 13 November, 2017; Accepted: 26 December, 2017

Abstract

Background: Human cytomegalovirus (HCMV) is a common virus that infects people of all ages. HCMV infection is an important and common complication in immunocompromised patients, especially transplant recipients. Antigenemia test and real-time PCR are one of the most common assays for diagnosis and monitoring of CMV infections. The aims of this study were to compare two common detection methods in order to identify clinically useful CMV infection in kidney transplant patients. **Materials and Methods:** One hundred and fifty peripheral blood samples from kidney transplant patients, including 78 men and 72 women aged from 4 to 73 years; with mean age of 36 years, collected during March 2016 to June 2016. Then samples were investigated for pp65-antigen on polymorphonuclear cells and HCMV DNA viral load on plasma and whole blood. **Results:** Out of 150 samples analyzed, HCMV DNA was detected in 47(31.33%) cases; with 26 (55.32%) and 21(44.68%) cases in men and women, respectively. The pp65 antigen was detected in 42(28%) casas; with 23 (54.76%) and 19 (45.24%) cases in men and women, respectively. Of the 150 samples, 42 (28%) were positive for both assays and 108 (72%)were negative. **Conclusion:** Our findings showed both tests were significantly correlated and can be useful for monitoring of CMV infection. Hence, higher viral loads have been found to be associated with increase of disease complication, Real- time PCR is more suitable. The findings merit more investigations involving larger numbers of samples.

Keywords: Human Cytomegalovirus, Real- time PCR, pp65 antigenemia, kidney transplant.

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Please cite this article as: Amirhosseini N, Shokri S, Mahmoudvand Sh, Moradli Gh A. Real-time PCR and pp65-antigen test for monitoring human cytomegalovirus infection in kidney transplant patients. Arch Med Lab Sci. 2017;3(3): 26-30.

Introduction

Human Cytomegalovirus (HCMV) is a member of the Herpesviridae family and is a ubiquitous virus with worldwide distribution (1, 2). Its seroprevalence in human is more than 70% and about 90% in developing countries (3, 4). HCMV is spread from an infected person by direct contact. People with CMV may shed the virus in body fluids, such as urine, saliva, blood, tears, semen, and breast milk. As well as, also organ transplants are one of the possible modes of transmission (5, 6). HCMV is one of the most common opportunistic virus that is controlled in individuals with normal immune system but established in immunocompromised patients or reactivated in transplant recipients (4, 7, 8). This infection occurs due to transmission from the transplanted organ, due to reactivation of latent infection, or after a primary infection in seronegative patients and can be defined as follows: latent infection, active infection, viral syndrome or invasive disease (9). "Active CMV infection occurs in 30-75 %

of transplant recipients, with a mortality rate of 5%" (4). Cytomegalovirus infection is one of the main infectious morbidities after renal transplant, leading to direct effects, such as disease, characterized by the viral syndrome or by the invasive disease, and to indirect effects, such as increased risk of acute rejection and chronic graft dysfunction (10).

The prevention of CMV disease is therefore a major goal in the clinical management of these patients. Cytomegalovirus disease can be prevented by prophylaxis or by preemptive therapy (9). Preemptive therapy reduces the incidence and the severity of the CMV disease; however, it depends on early laboratory identification of those at a high risk of disease (11). There are several diagnostic tests for detection of CMV, including serological tests, cell culture, antigenemia, polymerase chain reaction (PCR), immunohistochemistry, nucleic acid sequence-based amplification (NASBA) and hybrid capture assay (12-14). The key to efficient and effective management of CMV infection in these patients is to develop a highly sensitive and quantitative detection method capable of quantifying the CMV viral load and rapidly identifying patients at high risk of developing CMV disease, and monitoring the preemptive antiviral therapeutic strategies (15). Antigenemia and PCR tests use to for detection of active CMV infection by the identification of pp65 antigen-positive and CMV DNA in blood (16, 17). Antigenemia assay is widely used to monitor transplant recipients patients. This method aims to detect pp65 antigen expressed in CMV infected all nucleated cells (ANC) using a monoclonal antibody (18). This method has several limitations regarding its use in the clinical practice: it is a manual test, blood samples should be processed within 6–8 hours of collection, and high level of technical expertise is required to read the slides. leukopenia is one of the clinical symptoms of CMV infection. Hence, the evaluation the CMV pp65 antigen done in peripheral blood leukocytes, the interpretation of its results may be difficult in children and people with leucopenia. In this setting, qPCR is an alternative method for early detection of viral replication as it is not affected by leucopenia (19). Employing the sensitive, quick and specific diagnostic methods for early detection of CMV plays an important role in CMV prevention in transplant patients because the severity of CMV disease can reduce with using of antiviral drugs. The use of ganciclovir and some other antiviral drugs such as foscarnet and valganciclovir have reduced both the morbidity and mortality of HCMV disease (20). In this cross sectional study, a rapid commercial Cytomegalovirus pp65 antigenemia assay was compared to real-time PCR for detection and quantitation of CMV in clinical samples for kidney transplant patients.

Methods

All reported methods were carried out in accordance with the latest revision of the Declaration of Helsinki 1964

Patients. From March 2016 to June 2016, one hundred and fifty peripheral blood samples (including 72 females and 78 males) were collected from kidney recipients, admitted to Transplantation laboratory centers in Tehran city for CMV pp65 antigen and CMV DNA evaluation. A total of 150 blood samples were collected in citrate-anticoagulated vials.

HCMV pp65 antigenemia assay. This technique was performed within 4 hours of specimen collection. samples were processed for antigenemia within 4-5 hours, and the others were stored for quantitative plasma DNA determination. The determination of HCMV antigenemia was performed using a (CMV Groningen, Netherlands IQ Products, Brite TM Turbu) according to the manufacturer's instructions. The number of pp65-positive polymorphonuclear leukocytes (PMNL) was determined after staining with specific fluorescein isothiocyanate-labelled monoclonal antibodies and results were expressed as the number of fluorescing cells per 30,000 PMNL.

DNA extraction. Total nucleic acids were extracted from 200 µl of EDTA-anticoagulant whole blood with using of QIAamp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Then the extracted DNA was stored at -20°C.

HCMV DNA quantitation. HCMV DNA quantitation was carried out by a commercially available Nanogen Q-CMV Real Time Complete Kit (Nanogen Advanced Diagnostics, Torino, Italy) that has the UL123 gene as target region. This method was

performed according to the manufacturer's recommendations. PCR test were carried out as follows: pre-denaturation at 95°C for 15 min, 95°C for 5 s, 60°C for 20 s and 72°C for 15 s for 45 cycles. PCR reactions were performed using a Rotor Gene 6000 Instrument (Corbett Research, Doncaster, Australia).

Results

Out of 150 samples analyzed, HCMV DNA was detected in 47(31.33%) cases; with 26 (55.32%) and 21(44.68%) cases in male and female, respectively. Viral load in HCMV positive samples ranged from 71 copies/ml to 900×103copies/ml (Table 1). All specimens were positive for beta-globin DNA, confirming the quality of the DNA preparations. pp65 antigen was detected in 42(28%) casas; with 23 (54.76%) and 19 (45.24%) cases in male and female, respectively. Leukocytes count range in CMV pp65 antigen-positive were from 5×103/µl to 12×103/µl (Table 2). Of the 150 samples, 42 (28%) were positive for both assays and the rest were negative. five pp65-negative samples had HCMV DNA levels lower than 100 copies/ml. There was no sample that be pp65 antigen positive, but a Real time PCR negative. Comparison of results showed that no significant differences between the number of positive and negative samples obtained by two methods (P-value=0.61). Sensitivity and specificity of pp65 antigenemia assay and Real-time PCR method were 89.36% and 100%, respectively.

Discussion

Cytomegalovirus (CMV) is a genus of viruses in the Herpesviridae family and is ubiquitous in the human population (21). It is one of the main agents that infects up to 60−100% of people in adulthood (22). After primary infection, CMV is becomes dormant in its host and is not eradicated and may be reactivated under different conditions such as immunosuppression and inflammation (23, 24). CMV is a well-known pathogen in individuals with immunosuppressed including transplant recipients (25-27).

The incidence of CMV infection varies by the type of organ transplant, the serostatus of donor and recipient, and the prevention strategies used. After transplantation CMV may be activated and replicated, which might cause prolonged hospitalization, increasing the post-transplant costs and the threatening of new kidney and the life of recipient. Therefore, the prevention of CMV disease is a major goal in the management of kidney transplant patients and a reliable and sensitive laboratory techniques for diagnosis of CMV infection before the onset of symptoms will be pivotal (28, 29). Reducing the impact of CMV infection in transplant recipients is required by improving at diagnosis, prevention, and treatment. Despite these improvements in its diagnosis and therapy, CMV still has a major outcome on transplant patients (13). PP65 antigenemia and real-time CMV PCR are rapid diagnostic methods for detection of CMV infection and monitoring of transplant patients to reducing its outcomes (16, 30).

Table1. Diagnosis of CMV infection by Real-time PCR assay

	Number	Age		Gender		Viral load Copies/ml (Range)
		Male	Female	Male	Female	
Positive cases	47	32	31	26	21	71 -900×103
Negative cases	103	40	35	52	51	Not seen
Total	150			78	72	

Table2. Diagnosis of CMV infection by pp65 antigen assay

	Number	Age		Gender	
		Male	Female	Male	Female
Positive cases	42	34	32	23	19
Negative cases	108	37	36	57	51
Total	150			80	70

In this survey, we tried to show a good association between CMV and DNA measurements obtained using a commercial quantitative PCR assay and the pp65 antigenemia results. Our findings indicated that the sensitivity of pp65 antigenemia assay was lower than that real-time PCR test in screening and detection of CMV infection. This finding were consistent with the results of other studies that performed in Finland, Italy, Netherlands, United Kingdom, France and Iran (4, 8, 20, 31-35). Khansarinejad et al. suggests that the antigenemia method for monitoring HCMV reactivation could be substituted by the qPCR assay (20). Rangbar-Kermani et al. demonstrated that real time PCR assay is more sensitive for viral load determination, especially in some infection with low level of viral load and therefore, this test is an effective tool for starting preemptive therapy or monitoring the efficacy of anti CMV therapy (8).

Real time-PCR has been considered the gold standard test for early diagnosis of CMV replication and antiviral therapy and has several advantages such as fast method, easy to handle, sensitive assay, requires a small volume of sample, can be done on leukopenia patients, less influenced by the quality of sample. Also quantification of CMV by real-time PCR in body fluid has been shown to be useful in clinical contexts for monitoring of transplant recipients and antiviral therapy. In which higher viral loads have been found to be associated with increase of disease complication. Therefore, quantitative PCR-based assays might be too sensitive for clinical purposes (36).

In addition, this assay is expensive and its sample preparation is usually the most time-consuming (9). Therefore, the costs of this test are too high for implementation in public health hospitals.

In conclusion, the measurement of viral load by real-time PCR appears to be an important tool in the prediction, diagnosis of active CMV disease in immuno-compromised transplant subjects, for differentiating latent from active infection. Hence, pp65 antigen has acceptable sensitivity in detection of CMV infection in kidney transplant that this test can be replacement real time PCR in low income countries for screening in a population of patients.

Acknowledgments

This study was financially supported by Saveh Islamic Azad University.

Conflict of Interest

All authors declare no conflict of interest.

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