Iranian Journal of Virology 2016;10(2-3): 58-62

Short Communication

Detection of Cytomegalovirus (CMV) in Peripheral Blood Specimens in Patients before Keratoplasty

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Abstract

Cytomegalovirus is the most important pathogen affecting transplant recipients and causing significant morbidity and mortality. The prevalence of CMV infections in transplant recipient varies from country to country. The aim of this study was to determine the prognostic value of PCR assay for CMV detection in patients before Keratoplasty. A total of 23 patient samples enrolled in this study from March 2008 to Feb 2010. Based on the result the over all incidence of CMV infection was 39.1 percent (9 positive sample) in PCR assay. In this study, the plasma PCR assay proved to be sensitive and specific in order to detect CMV infections.

Keywords: Cytomegalovirus, CMV, Peripheral Blood Specimens, Keratoplasty

Introduction

Human CMV is a ubiquitous virus infection with worldwide distribution and Human CMV is transmitted from human to human; there is no animal reservoir (1-4). The effects of CMV infection in transplant recipients vary from clinically manifestations of active CMV diseases to allograft injury or loss (5, 6). Despite preventive antiviral medications, CMV infection is still a major cause of morbidity and mortality after bone marrow (BM) or kidney transplantation (5-8). Especially CMV – seropositive patients are at high risk for developing CMV infection (6, 9, 10). CMV disease occurs in the period 28 to72 day after transplantation. It can affect several organs, primarily the lung (interstitial pneunonitis) and the gut (6,9). Despite some improvements (new antiviral agent, combination therapy) the fatality rate for CMV pneumonia is still high (5,6,11) Considering CMV infection as the most significant risk factor for the occurrence of CMV disease, early detection of the virus is warranted in order to prevent the progression of the disease (9, 12-14). However, Unlike PCR other assays such as pp65 antigen detection sometimes shows false-negative results due to a low-level expression of the antigen in white blood cells in a small number of patients with definite disease (15, 16). The PCR-based technique has recently been applied to detect CMV - DNA in blood samples provides 100% sensitivity for the diagnosis of CMV infections.

Methods

Patient and samples. All transplant volunteer at the Gholhak laboratory of Tehran undergone laboratory-based buffy coat surveillance for CMV infection. A total of 23 patients, including Keratoplasty volunteer, were

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[DOI: 10.21859/isv.10.2.3.58]

enrolled in the present study from 2008 to 2010. A total 23 samples from these patients were assayed. At each sampling point two 4ml tubes of EDTA-treated blood were collected and Plasma was stored at -20°C after centrifugation for 10 min at 500×g.

Extraction of CMV DNA. DNA was extracted from 200µL of plasma and 1.6×106 PBL by using the manual phenol chloroform DNA extraction method according to the available protocol and suspended into the tube with 50 µL of elution buffer (17-19).

Primer design. The primers were selected and checked by using the two sets of Primer design software program (Gene runner & BioEdit). The conserve regions were amplified using the following primers so that the primers, Forward (5'-CGGTGGAGATACTGCTGAGGTC-3') and Reverse (5'-CAAGGTGCTGCGTGATATGAAC-3') amplified a 250 bp length product in a pp65 gene.

CMV PCR. CMV DNA was amplified by PCR technique and primer pairs targeted the glycoprotein B gene: 5'-CGGTGGAGATACTGCTGAGGTC-3'(P1, sense nucleotides 82494-82515), 5'-CAAGGTGCTGCGTGATATGAAC-3'(P2, antisense nucleotides 82729-82750) (26). PCR assay were done in 25 µl tube reaction as the following: 5 pl target DNA, 0.4 pmol of each primer, 0.2 pl deoxy nucleoside triphosphate (containing 25 mM dATP, dCTP, dGTP, and dTTP) and 1 unit of Taq polymerase (Perkin Elmer Cetus, Norwalk, California, USA). PCR was done as below: preheating at 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30s, 72°C for 30s and a final 72°C for 3min as a final extension step (26). The PCR products were electrophoresed in 1.5% agarose gel, stained with ethidium bromide, and visualized by an ultra-violate (UV) transluminator (figure 1). Positive and negative controls were tested in each reaction. As positive control, we used a previously confirmed CMV sample (26), and a sample consisting of distilled water was used as a negative control.

Statistical analysis. Mann–Whitney U-test was used for the statistical analysis to assess the association between antigenemia and PCR results. The results were expressed as medians and P values <0.05 were considered significant. P values were calculated using the chi-square test. Pearson correlation and linear regression were used to compare antigenemia and PCR CMV results. Data were analyzed by mean values and standard deviations for frequency and percentages for qualitative categorical variables. SPSS version 13.0 was used to analyze the data. A P value of _ .05 and 95% confidence intervals was accepted as statistically significant.

Result

All 23 samples were subjected to analyse by PCR test. The Results showed that 9 out of 23 samples were PCR-positive test. Among all the samples, 9 cases were PCR-positive and 20 cases have IgG antibody to CMV . Results have been summarized in Table1.

From our population, by sex and age hierarchy male and female in range 36-45 and 46-55 have more negative results for antigenemia test, and from other side mal in range 55-65 and female in range from 36-45 shown more positive results for this assay.

Positive results of PCR by sex and age showed similar aspect to antigenemia results, but mal in range from 2 6-35 and female by range 36-45 have more pcr negative results. By comparison, we assumed that, there was no relation between sex and age with tests results and the differences between observation not enough large to show accurate relationship.

Discussion

In transplant recipient, the sign of CMV infection are non specific (7, 8) and identification of CMV infections with reasonable accuracy by diagnostic tools are necessary (4-7, 9, 10, 13-15). Managing the CMV infections is a major consideration in providing care to transplant recipients and must be considered for its significant medical importance (7, 8). The polymerase chain reaction based techniques have recently be applied to the detection of various viral pathogens and these tests are recognized as

[DOI: 10.21859/isv.10.2.3.58]

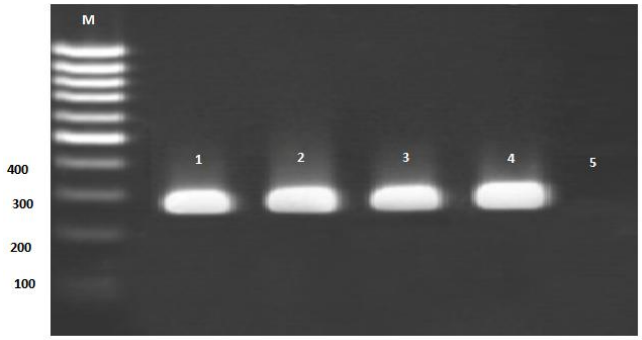

Fig. 1. Results of PCR for 250 bp of the CMV. M: marker; lane 1: positive control; lane 2,3,4: positive samples; lanes 5: negative control

rapid, simple, sensitive, reliable and cost effectiveness tests for detection of viral DNA or RNA(17-19). PCR based methods has been shown to be an extremely sensitive method for detecting small amount of latent HSV-1 genome as well as CMV DNA in peripheral blood leukocytes in renal transplant recipient(6, 9, 20). Despite preventive antiviral medications which have been used to decrease the incidence, CMV infection is still a major cause of morbidity and allograft rejections. Therefor, early diagnosis of CMV infection and its differentiation from other infections in allograft rejection remains of great importance (5, 21-23). In the present study a PCR technique was optimized and established for the rapid and early detection of HCMV in Keratoplasty volunteer and PCR assay proved to be more sensitive than antigenemia test in detecting CMV viraemia.

In effect by the plasma PCR assay, 174 of 187 episodes of CMV viraemia could be diagnosed, while the AG test was able to detect only 60 episodes. If the specificities of both assays are considered to be 100%, the sensitivities of the plasma PCR assay and AG were found to be 98.7% and 33.7%, respectively. In the current study 4 samples with discordant results were found to be plasma PCR assay negative and AG positive. Likewise, superior sensitivity of plasma PCR assay in comparison with the sensitivity of AG has been reported in different transplant settings (24, 25). In one study the number of subjects with a positive test result was significantly higher for AG than for the qualitative PCR assay and the AG result tended to turn positive earlier than PCR assay result

Table 1: summarized test Results

Test	Result	Number	Percent
PCR	+	9	39.1
CMV IgG antibody	+	20	86. 9
Only CMV IgG antibody	+	11	47.82
Both	+	9	39.1

did (24). Reason for such a discrepancy is not clear since the protocol used to perform AG appeared not to be substantially different from that followed in our study, and the same PCR method was used. Our data are also in contrast to those reported by Boeck and coworker, who found the sensitivity of the plasma PCR test, is similar to that of AG (24). That study used an in-house-designed PCR assay (26). In agreement with previous report, the AG result globally tended to turn negative earlier than the plasma result after the initiation of Ganciclovir treatment (24). In summary, the CMV plasma PCR assay is more sensitive than AG for the detection of CMV viraemia in transplant recipients.(27,28) In conclusion the advanced molecular techniques such as real-time PCR could be performed for diagnosis and monitoring of anti viral treatment(29,30).

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[DOI: 10.21859/isv.10.2.3.58]

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[DOI: 10.21859/isv.10.2.3.58]

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[DOI: 10.21859/isv.10.2.3.58]