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Development of PCR assay for early detection of CMV infection in renal transplant recipients

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ABSTRACT

Background: Cytomegalovirus (CMV) is an important viral pathogen in patients undergoing organ transplantation.
Objectives: We aimed to develop a molecular qualitative PCR assay for the detection of CMV DNA in clinical samples from renal transplant recipients.
Patients and Methods: Polymerase chain reaction (PCR) was performed for the assessment of CMV replication in two groups of renal transplant patients. Furthermore, the sensitivity of PCR for detection of CMV DNA from plasma relative to leukocyte was tested.
Results: CMV DNA was detected in 33 (25.9%) samples of 127 renal transplant patients. From 33 patients with positive PCR test, 20 patients had clinical symptoms and 13 (33.4%) of the patients had no clinical symptoms of disease. Also, the sensitivity of PCR assay for detection of CMV DNA from leukocyte relative to plasma is more.
Conclusions: The results suggest that The CMV PCR might be a useful tool for the early identification of patients at high risk of developing CMV disease. Furthermore the best sample for identify CMV infection is peripheral blood leukocytes (PBL).

▸ *Implication for health policy/practice/research/medical education:*
Due to importance of new diagnostic assays in renal transplant recipients, we suggest reading this interesting article for basic scientists like virologists and geneticicans who are studying viral infections.

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Background

Cytomegalovirus (CMV) is a highly prevalent infection around the world and like all herpes viruses, CMV establishes a state of persistent infection or probably true latency from which it is able to reactivate especially in immunocmpromised patients, such as pregnant women, patients with advanced HIV infection and allograft recipients (1). This virus is the most important cause of morbidity and mortality in organ transplant patients (2). An early and sensitive detection of active CMV infection is critical for directing antiviral therapy and assisting in patient management, in order to reduce morbidity and mortality of CMV disease. A virus culture method with detection of early antigen by immunofluorescence is sensitive and specific, but is laborious and unsuitable for routine processing of large numbers of specimens. Serological diagnosis of infection is unreliable in immunocompromised patients and may not be rapid enough to permit early treatment (3). New molecular tests have been developed which include the hybrid capture assay, RNA detection by nucleic acid sequence based amplification (NASBA), and DNA PCR (4). Detection of CMV DNA by PCR amplification contributes to rapid and early diagnosis of active CMV in organ-transplant patients to identify those at high risk of CMV disease before antigenemia becomes detectable (5).

Objectives

The aim of this study was to develop a qualitative in-house PCR assay for detection of CMV DNA from plasma and several PBL from 137 renal transplant recipients in comparison with a commercial kit.

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Patients and Methods

Patients and samples

Between January and November 2009, a cross-sectional study was conducted in 137 kidney transplant recipients. Patients were divided into two groups; 59 cases at the first week post transplantation and 78 cases at least 4 weeks after transplantation. A single blood sample was taken from each person for investigation of possible CMV infection. In this study, for comparison of sensitivity of PCR for detection of CMV DNA from plasma relative to leukocyte, corresponding EDTA whole blood and EDTA plasma specimens were obtained from that was in second group (fourth week after transplantation). Whole blood was stored using the Glycigel method and frozen at -70° C until DNA extraction. Among 137 patients, 93 were males and 34 females. The average mean of age of the patients was 41/1 years old.

DNA extraction from plasma

DNA was extracted from 250 µl plasma lysed in buffer (20 mM Tris-HCl, 1 mM EDTA,1% sodium dodecyl sulfate) and digested with proteinase K (2 mg/ml) at 37°C for 24 h, followed by sequential phenol and chloroform extractions and ethanol precipitation. The extracted was stored at -70°C until tested.

DNA extraction from Leucocytes

DNA was extracted from Glycigel preserved whole blood using Cunningham method (3). The extract was stored at -70° Cuntil processed.

In-house CMV PCR

CMV DNA was detected by PCR assay using pair primers associated with the glycoprotein B gene: 5'-CGGTGGAGATACTGCTGAGGTC-3'(P1, sense nucleotides 82494-82515), 5'-CAAGGTGCTGCGTGATATGAAC-3'(P2, antisense nucleotides 82729-82750). PCR reactions were done in 25 µl mixture reaction contained the following: 5 pl target DNA, 0.4 pmol of each primer, 0.2 pl deoxynucleosidetriphosphate stock (containing 25 mM dATP, dCTP, dGTP, and dTTP) and 1 unit of Taq polymerase (Perkin Elmer Cetus, Norwalk, California, USA).

PCR was performed with the following parameters: 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. The PCR products were isolated by 1.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized by an ultra violate (UV) transluminator. Positive and negative controls were included in each amplification. As positive control we used of a plasmid (PTZ57R) containing the 257bp region of CMV g B gene (6), and a sample consisting of distilled water was used as a negative control.

PCR assay according to commercial kit

In addition to in-house PCR, the second PCR assay was performed based on a commercial kit of Roche Company, according to the instructions of the manufacturer.

Results

Amplification of DNA with PCR disclosed that of 127 transplant patients, CMV DNA was detected in 33 (25.9%) samples. From 33 patients with positive PCR test, 20 patients had clinical symptoms of disease such as; fever, leucopenia, interstitial pneumonia and joint inflammation and 13 (33.4%) of the patients had no clinical symptoms of disease *(Figure 1)*.

Detection of HCMV from leukocyte

Leukocyte samples from 10 renal transplant recipients, corresponding to their plasma samples were tested for the presence of CMV by the PCR assay. From 10 Leukocyte samples, PCR assay was positive for 4 patients, while their corresponding plasma had negative result. The patients that had CMV infection were similar to those who did not have infection with regards to age and sex. There was a good association between the in-house PCR and commercial PCR kit assays in all of the patients.

Sensitivity and specificity of the PCR assay

We determined the sensitivity of the PCR assay on a plasmid of known molecular length. The plasmid PTZ57R, which contains the 257 bp region of CMV gB gene, was serially diluted and subjected to 30 cycles of amplification. As little as 1 fg of plasmid DNA was amplified and detected (the result is not shown). The specificity of the assay was examined by amplifying DNA from a number of other herpes viruses including herpes simplex virus type 1 or 2, Varicella-Zoster virus, Epstein-Barr virus. The PCR assay did not cross-react with EBV, VZV, HSV1 and HSV2.

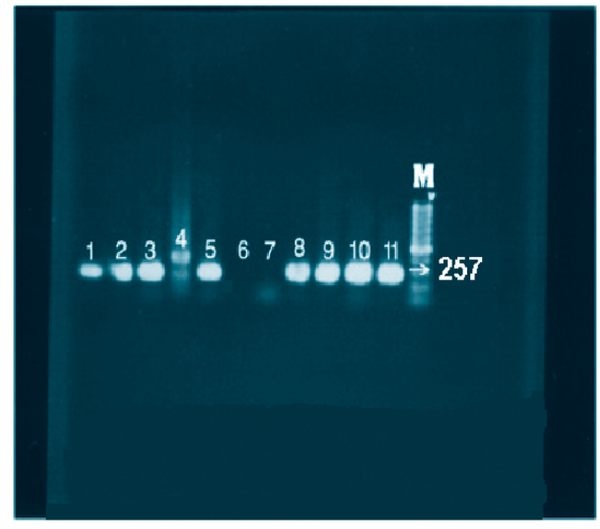

Figure 1. Ethidium bromide-stained agarose gel electrophoresis: identification of CMV DNA with p1 and p2 primers. Lane M,100 bp DNA marker (Sinagen, Iran) lane 1, positive control lane 2,3,5,8,9,10 and 11, positive patient samples lane 4 and 7, negative patient samples lane 6, negative control. The size of gB product is 257 Bp.

Discussion

CMV infection is a recognized cause of morbidity and mortality in organ transplant patients. Early detection of CMV by sensitive assays could be useful for the treatment and monitoring of transplant recipients at high risk of developing CMV disease. Currently, the quantification of pp65 antigen is used as a tool to predict which patients will develop CMV disease. However, this assay has low specificity. It has demonstrated that the amplification of CMV DNA by PCR assay,

is useful to establish a diagnosis and to monitoring of CMV disease in these group of patients (7). There have been many studies evaluating the clinical utility of PCR-based assays for the detection of CMV DNA in the plasma or PBL. Some of them reported that diagnostic sensitivity of a PCR assay may be different according to oligonucleotide primers sets and also kind of clinical sample that used (3, 8-11).

In our study, the gB oligonucleotide primer sets which amplify a fragment of the region that encodes the glycoprotein B were chosen. These primers were designed from the pattern strains AD169 and Towne; they amplify genome regions that are considered as a high degree of conservation of the epitope between the clinical isolates (12-14). Several comparative studies have demonstrated that gB primer sets have high diagnostic sensitivity of HCMV infection among clinical strains (11, 15). Some published reports have suggested that in order to monitor CMV infections in immunosuppressed patients, the sample of choice next to plasma and serum is leukocyte (16-19). Although small in terms of patient numbers, our results demonstrated that there is difference between leukocyte and plasma with regard to the time of detection of CMV DNA by PCR prior to the onset of symptomatic CMV infection. Thus, leukocyte PCR assay seemed to be significantly more sensitive than plasma PCR. The sensitivity of the assay and earlier detection can be clinically important because start of antiviral treatment at this earlier time could be important for both better efficacy and reduced toxicity (20). However, a problem with this technique is that it may detect latent CMV DNA in these patients without active viral replication. To avoid this problem, we used primer sets belonging to a conserved gene that amplify the late regions of the CMV genome. Therefore, this assay is too sensitive and it may detect CMV DNA in asymptomatic patients who never develop CMV disease. As a result, we suggest that CMV monitoring in PBL could be superior to assays in plasma or serum. However, this remains to be virologically and clinically evaluated in future studies. In conclusion, we have shown that the detection of CMV-DNA by in house PCR is a very sensitive assay to diagnose CMV disease in renal transplant recipients and correlate with commercial kit of Roche Company. Thus, our in-house nucleic acid amplification methods are especially suitable for detection of low-level virus replication. However, despite its sensitivity, the quantitative PCR could be another alternative in the diagnosis of CMV infections and in the monitoring of the response to antiviral treatment as this may be a better predictive tool. Also, we have shown that PCR with PBL offers the advantage of providing an earlier detection compared to the time to detection with plasma which makes it a more optimal marker for preemptive therapy.

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Conflict of interest

None daclared.

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References

1. Kulkarni A, Westmoreland D, Fox JD. Molecular-based strategies for assessment of CMV infection and disease in immunosuppressed transplant recipients. *Clin Microbiol Infect*. 2001;**7**(4):179-86.
2. Tang W, Elmore SH, Fan H, Thorne LB, Gulley ML. Cytomegalovirus DNA measurement in blood and plasma using Roche LightCycler CMV quantification reagents. *Diagn Mol Pathol*. 2008;**17**(3):166-73.
3. Cunningham R, Harris A, Frankton A, Irving W. Detection of cytomegalovirus using PCR in serum from renal transplant recipients. *J Clin Pathol*. 1995;**48**(6):575-7.
4. Lopez-Rocafort L, Brennan DC. Current review of cytomegalovirus in renal transplantation. *Minerva Urol Nefrol*. 2001;**53**(3):145-58.
5. Prosch S, Kimel V, Dawydowa I, Kruger DH. Monitoring of patients for cytomegalovirus after organ transplantation by centrifugation culture and PCR. *J Med Virol*. 1992;**38**(4):246-51.
6. Ghaffari SH, Obeidi N, Dehghan M, Alimoghaddam K, Gharehbaghian A, Ghavamzadeh A. Monitoring of cytomegalovirus reactivation in bone marrow transplant recipients by real-time PCR. *Pathol Oncol Res*. 2008;**14**(4):399-409.
7. Caliendo AM, Schuurman R, Yen-Lieberman B, Spector SA, Andersen J, Manjiry R, et al. Comparison of quantitative and qualitative PCR assays for cytomegalovirus DNA in plasma. *J Clin Microbiol*. 2001;**39**(4):1334-8.
8. Abecassis MM, Koffron AJ, Kaplan B, Buckingham M, Muldoon JP, Cribbins AJ, et al. The role of PCR in the diagnosis and management of CMV in solid organ recipients: what is the predictive value for the development of disease and should PCR be used to guide antiviral therapy? *Transplantation*. 1997;**63**(2):275-9.
9. Wolf DG, Spector SA. Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. *Transplantation*. 1993;**56**(2):330-4.
10. Garrigue I, Doussau A, Asselineau J, Bricout H, Couzi L, Rio C, et al. Prediction of cytomegalovirus (CMV) plasma load from evaluation of CMV whole-blood load in samples from renal transplant recipients. *J Clin Microbiol*. 2008;**46**(2):493-8.
11. Distefano AL, Alonso A, Martin F, Pardon F. Human cytomegalovirus: detection of congenital and perinatal infection in Argentina. *BMC Pediatr*. 2004;**4**:11.
12. Coyle PV, Wyatt DE, Ong GM, Maxwell AP, McCaughey C, O'Neill HJ. A nested primer set targeting the cytomegalovirus glycoprotein B gene. *J Clin Virol*. 2002;**25**(1):95-6.
13. Barbi M, Binda S, Primache V, Caroppo S, Dido P, Guidotti P, et al. Cytomegalovirus DNA detection in Guthrie cards: a powerful tool for diagnosing congenital infection. *J Clin Virol*. 2000;**17**(3):159-65.
14. Darlington J, Super M, Patel K, Grundy JE, Griffiths PD, Emery VC. Use of the polymerase chain reaction to analyse sequence variation within a major neutralizing epitope of glycoprotein B (gp58) in clinical isolates of human cytomegalovirus. *J Gen Virol*. 1991;**72**(Pt 8):1985-9.
15. Barber L, Egan JJ, Turner AJ, Guiver M, Woodcock AA, Yonan N, et al. The development of a quantitative PCR ELISA to determine HCMV DNAaemia levels in heart, heart/lung and lung transplant recipients. *J Virol Methods*. 1999;**82**(1):85-97.
16. Gerna G, Baldanti F, Zella D, Furione M. Detection of human cytomegalovirus DNA: how, when and where? *Scand J Infect Dis Suppl*. 1995;**99**:11-5.
17. Razonable RR, Brown RA, Wilson J, Groettum C, Kremers W, Espy M, et al. The clinical use of various blood compartments for cytomegalovirus (CMV) DNA quantitation in transplant recipients with CMV disease. *Transplantation*. 2002;**73**(6):968-73.
18. Boeckh M, Gallez-Hawkins GM, Myerson D, Zaia JA, Bowden RA. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation: comparison with polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. *Transplantation*. 1997;**64**(1):108-13.
19. Gerna G, Furione M, Baldanti F, Sarasini A. Comparative quantitation of human cytomegalovirus DNA in blood leukocytes and plasma of transplant and AIDS patients. *J Clin Microbiol*. 1994;**32**(11):2709-17.
20. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996;**88**(10):4063-71.