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Short Communication

Quantitative Analysis of CMV-DNA Load in Renal Transplant Recipients Using Real-Time PCR

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Primary infection with CMV is controlled in individuals with normal immune system, but when immune suppression occurs, CMV can reactivate and grow to high titer. The ability of CMV to remain latent after infection, contributes a great deal to serious CMV diseases (1). After transplantation the severe immunosuppressive regimes use to prevent rejection of the transplant, make the recipients prone to severe CMV disease. Infection with cytomegalovirus (CMV) is a significant cause of morbidity and mortality in renal transplant recipients, (up to 60% of primary infection) (2). The detection of CMV-DNA in plasma suggests active viral replication and spread of the virus from the leukocyte into the plasma (3). Viremia is only marker of active CMV infection. The ability to amplify CMV-DNA by PCR (qualitative and quantitative) has become a valuable diagnostic tool for detection of CMV in the early stages of infection prior to the disease (4). Although antigenemia is the gold standard in diagnosis and monitoring of infection, there is concern that some infections with low level of viral load that cannot be detected by antigenemia.[5]There is a variation in viral load results among different in house-developed CMV PCR quantitative (qn) assays (6). Therefore, we need to use a standard assay to reduce the variation in viral load results. The aim of this study was to investigate CMV-DNA viral load in active CMV infected renal transplant patients using LC Real -Time CMV PCR technique (Roche system, version 2). In this cross sectional study, we investigated CMV DNA viral load in 30 active CMV infected renal transplant recipients (15 females and 15 male and mean age 42.9 years), who referred to Iranian Blood Transfusion Organization (IBTO) research center during 2008 to2009. A written informed consent was taken from renal transplant recipients prior to enrolment into the study. We used plasma negative sample and internal control to prevent false positive results and to control DNA extraction procedure in each qn and ql PCR run. Viral DNA was extracted from 0.2ml plasma by using of QiAamp DNA blood mini kit (Qiagen, Germany) and Real- Time PCR (q RT-PCR) was performed by Artus CMV LC PCR kit based on manufacture (5) instruction. This kit is designed on the basis of specific prob. The correlation between the number of antigen-positive cells and level of CMV-DNA in plasma was analyzed by Pearson correlation test. The average of CMV DNA viral load was 7.61×10^7copies/ml (min. load: 1.38×10^2 copies/ml and max load: 1.9×10^9 copies/ml). The number of pp65-positive cells in 150000 cells was between 0 and 500 (mean value=43.67) positive cells. We observed low correlation between the number of pp65-positive cells and the titer of CMV DNA copies($r=.69$, $p<0.05$ by Pearson correlation). In this present study, we observed correlation between two CMV assays, quantitation PCR and antigenemia. This result is in agreement with many studies that have been reported

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correlation between two methods (7-9). In one patient who had positive results in both assays (82 cells/150000 cells and 23775 copies/ml CMV-DNA), CMV antigen was not detected but CMV-DNA was detected(300 copies/ml). Two other patients had positive results in quantitative PCR assays (138 and 585copies/ml), but CMV antigen was not detected by antigenemia assay. The number of positive results in real time PCR (q RT-PCR) assay was greater than antigenemia assay. This result is in agreement with other researches, and suggesting that real time PCR (Q RT-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 (7-12). It seems that the main advantages of CMV-DNA quantitation by using of real-time system is detection of viral genome with specific primers and probes, but in antigenemia assay, we can detect the viral protein. Because of existing variation in qn results in different laboratories, further studies are need to compare CMV antigenemia titer as a routine assay with viral load results for viral load cut-off value for CMV. This may result in establishing a preemptive therapy to increase the clinical utility of this assay. In this study, we had some limitation such as in availability of patients to obtain sequentional samples for monitoring and compare viral loads on the basis of two assays. In conclusion, in view of the toxic side effects of available antiviral agents, use of rapid and sensitive assays such as antigenemia and qn PCR is very important for management of CMV infection and disease. This reported qRT was sensitive method for quantification of CMV DNA in plasma of these patients and can be a useful tool for these purposes, and can be used as complementary test.

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