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Original Article

Utility of quantitative real time PCR in detection and monitoring of viral infections in post renal transplant recipients

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ABSTRACT

Background: Viral infections cause significant morbidity and mortality in post-transplant period. A highly sensitive and specific detection tool if used may help in early diagnosis and better management in these patients. The study aimed to assess the utility of quantitative real-time polymerase chain reaction (qRT-PCR) as a diagnostic and monitoring tool for viral infections in post renal transplant patients.
Methods: A quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect EBV and CMV infection in 50 patients on 1st, 2nd, 3rd, and after 6 months of renal transplantation.
Results: CMV infection was found in 34%, EBV in 28% of recipients, and 17% showed dual infection. Viruses were detectable after the first month of transplantation followed by symptomatic infections within first three months of follow-up, with diarrhea being the commonest symptom. These patients were also at high risk for developing other infections. Anti-thymocyte globulin (ATG) induction was a definitive risk factor for CMV/EBV infection in post operative period.
Conclusion: Renal transplant patients frequently develop one or more viral infections at a time. Regular monitoring with qRT-PCR and prompt antiviral therapy with reduction in immunosuppression may be an ideal approach for management of these patients.

1. Introduction

Advances in surgical and organ preservation techniques and improved immunosuppressant therapy together have resulted in evolution of renal transplantation as a treatment of choice in patients of end-stage renal disease. Though, immunosuppression is the key to success of transplantation it results in several adverse outcomes and unpredictable long term graft survival. Heavy doses of immunosuppressive drugs invite various types of complications amongst which the opportunistic infections are most common. Since, viruses get easy entry in to the host they are the usual pathogens responsible for infection induced morbidity and mortality in renal transplant recipients. Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) are the commonest offenders though, other viruses e.g. BK polyomavirus, herpes simplex virus, hepatitis viruses, human T cell lymphotropic virus-1, etc. are also encountered sometimes.[1]

CMV, a member of Herpes virus family infects 20–60% of transplant recipients.[2] It has both direct and indirect effects on renal allograft.[3] CMV infection may present as asymptomatic viremia or with fever and neutropenia – often a 'flu-like' illness with myalgia and fatigue. It suppresses the immune functions and predisposes to the development of secondary opportunistic bacterial and fungal infections. Another indirect effect of CMV viremia is graft rejection.

EBV is another reprobate associated with graft dysfunction and premature graft loss.[4] It may also result in subsequent development of EBV-related post-transplant lymphoproliferative disorders (PTLD). EBV reactivation occurs in adults in different clinical situations associated with chronic immunosuppression and is likely to be underestimated in transplant patients.[5]

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Despite being a major health problem, the magnitude of viral infections in India and their effects on transplant recipients have not been studied thoroughly. Most studies in the past have used antigenemia determined by an enzyme immunoassay (EIA), an indirect method, to investigate the viral infections. An EIA moreover has inherent intra- and inter-laboratory variability in the results thereby affecting comparability and in turn clinical practice. Robust and more sensitive screening or diagnostic measure like actual viral load using quantitative real time PCR (qRT-PCR) has an edge over serological methods and is now considered to be a method of choice for identifying viral infection. The qRT-PCR not only gives a better idea about impact of viral infections after transplantation but may also help in their timely detection and management of graft recipients thereby improving the outcome.

Also, little is known about influence of immunosuppressive drugs or induction therapy on risk of infection or reactivation of virus and their association with rejection episodes. Few studies utilizing serological assays for defining viral infections have been recently published but there is need to redefine the actual incidence of viral infections and their impact on transplant outcome. Studies on quantitative analysis of CMV and EBV co-infection in patients of renal transplant are meager and further validation of earlier similar studies is needed.[6,7] We therefore aimed to analyze the role of two most common viral infections, the CMV and EBV in renal transplant outcome, by quantification of EBV and CMV DNAemia in peripheral blood of renal transplant recipients in different time intervals using qRT-PCR.

2. Methods

In this prospective longitudinal observational study, conducted in the departments of Renal Transplant Surgery and department of Immunopathology, a total of 50 patients undergoing renal transplantation were included and followed up for a duration of 6 months after renal transplant. A written informed consent was obtained from the patients as per guidelines and the study was approved by the Institute's Ethics Committee.

Clinical details and demographic data were collected. Baseline investigations were carried out and tests for checking CMV, hepatitis B, C and human immunodeficiency virus (HIV) seropositivity were done in both patients and the donors at the time of preparation for transplantation. Immunosuppressive medications in transplant recipients were started 24 h prior to transplantation consisting of a combination of either tacrolimus or cyclosporine A with either mycophenolate mofetil (MMF) or azathioprine (AZT). In addition, unrelated and spousal transplants were given IL-2 receptor antagonist (Basiliximab 20 mg, 2 doses) or antithymocyte globulin (ATG; Thymoglobulin 3–3.5 mg/kg over 3 days) as an inducing agent. After achieving a stable graft function, as determined by daily urine output, serial creatinine measurements and free of any evidence of active infection, patients were discharged from the hospital. Recipients were called up for weekly follow-up on outpatient basis till the end of third month after transplantation and thereafter at the end of 6 months. Data regarding graft dysfunction and immunosuppression were collected at the time of discharge and during follow-up intervals.

Monitoring for CMV by qRT-PCR was done after 1, 2, 3 and 6 months and for EBV at 1, 3, and 6 months after renal transplantation. Additional tests for CMV and EBV by qRT-PCR were done if patient presented with symptoms suggestive of viral infection e.g. fever, flu like symptoms, decreased blood counts, hepatitis or other signs of organ specific manifestation and graft dysfunction. None of the patients received any primary prophylaxis for CMV or EBV after transplantation.

Table 1
Sequence details of primers used for CMV and EBV DNA quantification.

Virus	Target gene	Specific forward and reverse primers
CMV	CMV gB	F 5′-GCACCATCCTCCTCTTCCT-3′ F 5′-GGCCTCTGATAACCAAGCC-3′
EBV	BNRF1 p143 EBVp143	F 5′-GGAACCTGGTCATCCTTTGC-3′ R 5′-ACGTGCATGGTTAAT-3′

CMV and EBV viral load were measured (in 50 cases and 36 cases respectively) by qRT-PCR system (LightCycler LC480 Real Time PCR system, Roche applied Science, USA) using TaqMan probe chemistry for absolute quantification. In brief, the genomic DNA was extracted from 250 μl of EDTA blood using AxyPrep DNA extraction kit (Axygen Biosciences, USA) and the protocol was followed as per manufacturer's instructions. The primers for CMV were designed from the CMV glycoprotein B (gB) gene (strains) and for EBV from nonglycosylated membrane protein BNRF1 p143 (Table 1).

The genomic DNA thereafter was amplified using primer-probe and mastermix (Roche Diagnostics GmbH, Roche Applied Science, Germany) containing Taq DNA polymerase, reaction buffer, and deoxynucleotide triphosphates (dNTPs). The reaction was initially denatured for 5 min at temperature of 94 °C. The annealing and extension temperatures were 58 °C and 72 °C and number of cycles was 40. The exact number of copies/ml blood was calculated by extrapolation from the standard curve obtained using commercially available standards (CMV strain AD169). Serial dilutions were run on LightCycler and a linear assay was obtained across 6 orders of magnitude, sensitive for 10^1–10^8 copies/ml, and multiplied by the multiplication factor of 600 (lowest range of detection of number of copies/ml).

After detection of viral infection, standard protocols for the management of CMV/EBV infection in transplant recipients were followed. Initially patients were treated with 5 mg/kg of Ganciclovir with concurrent reduction in immunosuppression till viral load was negative or for a minimum of 3 weeks. This was followed by 900 mg of Valganciclovir prophylaxis for up to 3 months.

3. Statistical analysis

For time fixed variables, continuous data were assessed using *Student t test* or *Mann–Whitney U test* depending on data distribution. Receiver operating characteristic (ROC) plot analysis was used to determine a cutoff of significant viral load. Descriptive statistics for population profiles, frequency and percentage for categorical data and mean and standard deviation for continuous data were used.

4. Results

4.1. General characteristics of study group

Among the patients there were 43 males (86%) and 7 females (14%). Mean age was 35.1 ± 10.9 years (range 12–58 years). Chronic glomerulonephritis was most common primary disease (42/50; 84%) followed by IgA nephropathy and adult polycystic kidney disease (3/50; 6% each) and diabetic nephropathy (2/50; 4%). Twenty one patients (42%) had living, related donor; 23 (46%) had living unrelated donors and deceased donors were used for another 6 (12%) patients. Among the donors, 18 were males (36%) and 32 females (63%). Mean age was 40.7 ± 12.7 years. All except one were serologically negative for hepatitis B and C. All the patients as well as donors sera were non-reactive for HIV. Pre transplantation CMV seroprevalence in the patients was 96% and 98% among the donors ($donor^+$/$recipient^+$ 48 patients, $donor^+$/$recipient^-$ and $donor^-$/$recipient^+$ 1 patient each) (Table 2).