Detection of Reactivation of Cytomegalovirusin Renal Transplant Recipients

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Abstract:Background: Cytomegalovirus infection in renal transplant recipients is a major clinical problem that may cause significant morbidity and mortality. Infection can occur as a result of reactivation of latent virus or new infection from donor tissues. Objectives: To assess the incidence of cytomegalovirus (CMV) reactivation, and to determine the predictive factors for CMV reactivation in renal-transplant patients, also to compare CMV-DNA amplification using qRT-PCR with serologic assays of CMV-IgM antibodies to detect CMV reactivation.**Study design**: Sixty patients were included in this study. They were classified into 3 groups based on the post transplantation period during which the study was performed. ELISA was used to detect the pre-transplantation CMV serostatus for the donor and the recipient as well as the recipient post transplantation CMV serology. CMV DNAemia was assessed by qRT-PCR first on whole blood (WB). Whenever a positive result was obtained; the assay was then performed on plasma to detect the difference between them.**Results**: CMV reactivation occurred in two patients following the treatment of their rejection episode and was detected by qRT-PCR using whole blood and not in plasma.**Conclusion**: Cytomegalovirus reactivation was not high in the studied patients, which may be due to the presence of pre existing immunity in the form of neutralizing antibody. The treatment of an episode of acute allograft rejection was the most important risk for CMV reactivation within the first year posttransplantation. qRT-PCR is an important tool in predicting subsequent or ongoing disease, while detection of anti CMV-IgM antibodies is not sensitive enough for diagnosis.

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1. Introduction

Human cytomegalovirus**(**CMV**)** is a ubiquitous Herpesvirus that persists for the life of the host following initial infection [(1)].Cytomegalovirus (CMV), which frequently causes latent asymptomatic infection in healthy adults, may evade immune surveillance in immune compromised patients and start to replicate [(2)]. After transplantation**,** CMV infections can occur as a result of the reactivation of an existing latent infection in the recipient, or a primary infection in a previously CMV-naive individual usually from the donor's organ or blood [(3)].

Cytomegalovirus virus infection and disease are the major infectious complication in renal allograft recipients, leading to increased patient mortality, graft loss, risk for acute rejection episodes and impaired renal function. The donor CMV seropositivity, the absence of CMV prophylaxis and the occurrence of acute rejection before CMV reactivation and its treatment with antilymphocyte antibodies, are all considered as independent risk factors associated with CMV reactivation within the first year after kidney transplantation [(4)].

Two approaches for CMV disease prevention are currently considered acceptable: universal prophylaxis and preemptive therapy. Prophylaxis is associated with the risk of late-onset CMV disease and ganciclovir resistance. By contrast, preemptive therapy requires frequent monitoring of CMV activity using sensitive methods such as polymerase chain reaction (PCR)[(5)].

Early detection of CMV DNA in the blood is of great importance to identify those patients who are at risk of infection and disease. Recent techniques such as qRT-PCR have improved the monitoring of CMV infection after kidney transplantation. The measurement of viral load by qRT-PCR appears to be an important tool in the prediction/diagnosis of CMV disease, and for differentiating latent from active infection and also for monitoring anti-CMV therapy [(6)].

Detection of CMV-IgM antibodies by various immunoassays is not sensitive enough for diagnosis and cannot be used for CMV monitoring during the active period in renal transplant recipients [(7)].

The aim of this work was to detect reactivation of CMV in renal transplant recipients and to compare CMV serology, the method routinely used, with qRT-PCR which is an important tool in predicting subsequent or ongoing disease. Also to determine the predictive factors for CMV reactivation in the Egyptian renal transplant patients, who will require

more frequent CMV monitoring in their post transplantation period.

2. Patients and Methods:

Patients:

The study was performed at Cairo University Hospitals (New Kasr El Aini Teaching Hospital and King Fahd during the period from June 2008 to August 2010.

Sixty patients were enrolled in the study, 42 of whom were males and 18 were females and age ranging from 12 to 51 years. All patients had undergone renal transplantation for treatment of end stage renal disease of different aetiology. All patients were subjected to chronic regular haemodialysis of different duration with history of blood transfusion. All patients were informed about their involvement in the study and they gave their consents.

The patients were classified into three groups based on the post transplantation period during which the study was performed: Group I: The laboratory tests were performed during the first postoperative month (the early post-transplantation period). It included 20 patients; 14 males and 6 females and ages ranging from 25 to 52years. Group II: The laboratory tests were performed between the 2 nd and 6 th post transplantation months. It included 20 patients; 16 males and 4 females and ages ranging from 22 to 48 years. Group III: The laboratory tests were performed during the late post transplantation (more than 6 months postoperative). It included 20 patients; 12 males and 8 females and ages ranging from 12 to 35 years.

The immunosuppressive regimen was based on triple therapy by calcineurin inhibition with cyclosporine (CsA) or tacrolimus, prednisolone and nucleoside antagonism with mycophenolatemofetil (MMF). All acute rejection episodes were treated by methylprednisolone pulse therapy. Steroid-resistant rejection was treated by antibody therapy, antithymocyte globulin, or orthoclone (OKT3).

All patients were subjected to: Detailed history with special stress on history of fever, jaundice and colitis. The following data were obtained from the patients' files:

a. Routine laboratory and biochemical tests in the form of: full blood picture, blood glucose level, lipid profile, blood urea, serum creatinine and blood electrolytes.

b. Presence or absence of blood borne viruses: HBV, HCV and HIV.

c. The pre-transplantation CMV serostatus for the donor and the recipient in the form of CMV IgG and IgM detection by enzyme linked immunosorbant assay (ELISA).

Post transplantation detection of CMV IgG and IgM was done using RadimcitoIgG and IgM EIA (Radim Spa Roma, Italia). Real-time PCR assay for detection and quantification of CMV DNA was done using the light cycler instrument.

Quantitative Real-time PCR was first done on whole blood (WB), whenever a positive result was obtained; the assay was then performed on plasma todetect the difference between them. This was done using [MagNA Pure LC DNA isolation kit I, Instruction manual; version November 2005 Cat. N:03730964001 Roche Diagnostics, Mannheim Germany].

3. Results:

Sixty patients were enrolled in this study. The characteristics of the studied groups are shown in table (1)

Post-operative screening for anti CMV IgG and IgM by ELISA test system: All patients (100%) were anti -CMV IgG positive and only 3 patients (5%) were anti-CMV IgM positive. Two out of the three patients were in group I and only one patient was in group II. They had negative CMV PCR and did not suffer from any post transplantation rejection episode during the period of the study. The distribution of IgM positive in the different study groups is shown in figure (1)

Real-time PCR performed on whole blood showed that CMV DNA was detected in two patients 2/60 (3.33%) who showed a positive amplification plot. The remaining fifty eight patients (96.7%) showed absence of CMV DNA in the form of a negative amplification plot. The test was done on the plasma of the two patients whose whole blood was positive for CMV DNA; using the same set of primers and probes. Both patients showed a negative amplification plot for CMV DNA.

Both patients with positive CMV real-time PCR were males and belonged to Group II 2/20 (10%) and the cause of the ESRD was diabetic nephropathy in one of them and pyelonephritis in the other. Both patients presented with fever, malaise, anorexia and one of them had laboratory evidence of impaired liver function in the form of elevated transaminases. Both patients were HCV positive. Both patients gave positive history of previous dialysis for more than 1 year and only one of them gave history of previous blood transfusion. Both patients had negative anti CMV IgM in the post transplantation screening test. The sensitivity and specificity of anti-CMV IgM to detect CMV reactivation post-transplantation (using positive RT–PCR as true positive) were 0% and 94.8% respectively. While the positive predictive value was 0% and the negative predictive value was 96.4%.

Both patients received CsA in their immunosuppressive regimen, which is statistically insignificant percentage of those who received CsA (2 /52 patients i.e. 3.84 %) p > 0.05. Both patients were treated for rejection episode

Patients with rejection episodes:

Post transplantation rejection episodes in the form of impairment of kidney function with elevation of serum level of both urea and creatinine were found in 6 patients (6/60 i.e. 10%), 5 out of them belonged to group II (5/20 i.e. 25%) and only one patient belonged to group I (1/20 i.e. 5%). The characteristics of patients who suffered from rejection episodes is shown in table (2).

Two out of the 5 patients in group II were positive for CMV- DNA by PCR post transplantation (2/5 i.e. 40%), constituting 33.33% (2 /6) among all cases with rejection episodes, which is statistically significant p = 0.00 (p< 0.05). Post transplantation anti CMV IgM was negative in the six patients. Four out of the six patients suffering from rejection episodes were also positive for HCV; three of them belonged to group II and only one patient belonged to group I.

Table (1): Characteristics of patients in the studied groups

	Group I (n =20)		*Group II (n =20)*		*Group III (n =20)*		*Total (n = 60)*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Cause of transplantation:								
• Hypertension	*11*	*55%*	*11*	*55%*	*11*	*55%*	*33*	*55%*
• Diabetic glomerulonephritis	*7*	*35%*	*5*	*25%*	*6*	*30%*	*`8*	*30%*
• Pyelonephritis	*2*	*10%*	*4*	*20%*	*3*	*15%*	*9*	*15%*
Prior renal dialysis:								
• > 1 year	*19*	*95%*	*18*	*90%*	*13*	*65%*	*50*	*83.33%*
• < 1 year	*1*	*5%*	*2*	*10%*	*7*	*35%*	*10*	*16.67%*
Previous Blood transfusion	*17*	*85%*	*11*	*55%*	*18*	*90%*	*46*	*76.66%*
Co-infection with:								
• HCV	*6*	*30%*	*5*	*25%*	*5*	*25%*	*16*	*26.6%*
• HCV & HBV	*2*	*10%*	*0*	*0%*	*1*	*5%*	*3*	*5%*
Pre-operative CMV IgG:								
• Positive	20	*100%*	20	*100%*	20	*100%*	60	*100%*
• Negative	0	*0%*	0	*0%*	0	*0%*	*0*	*0%*
Pre-operative CMV IgM:								
• Positive	0	*0%*	0	*0%*	0	*0%*	*0*	*0%*
Post-operative CMV IgG:								
• Positive	20	*100%*	20	*100%*	20	*100%*	60	*100%*
• Negative	0	*0%*	0	*0%*	0	*0%*	*0*	*0%*
Post-operative CMV IgM:								
• Positive	2	*10%*	0	*0%*	0	*0%*	*2*	*3.33%*
• Negative	18	*90%*	20	*100%*	20	*100%*	58	*96.67%*
Positive post-operative Real-time PCR for CMV on whole blood.	*0*	*0%*	*2*	*10%*	*0*	*0%*	*2*	*3.33%*
Positive post-operative Real-time PCR for CMV on plasma.	*0*	*0%*	*0*	*0%*	*0*	*0%*	*0*	*0%*
Rejection episodes	*1*	*5%*	*5*	*25%*	*0*	*0%*	*6*	*10%*
Treatment regimen with triple therapy based on:								
• CsA	*18*	*90%*	*17*	*85%*	*17*	*85%*	*52*	*86.67%*
• Tacrolimus	2	*10%*	*3*	*15%*	*3*	*15%*	*8*	*13.33%*

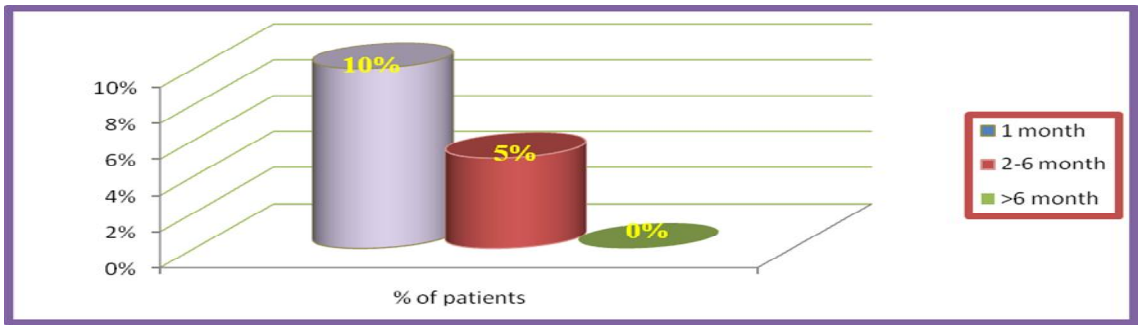

Figure (2):Distribution of positive post transplantation anti CMV Ig M in the studied groups

Table (2) The characteristics of patients who suffered from rejection episodes:

Patients	Group	Co-infection with HCV	CMV-DNA by real-time PCR	Post-transplantation anti CMV IgM	CsA or Tacrolimus based triple therapy
First	I	HCV	negative	negative	CsA
Second	II	HCV	positive	negative	CsA
Third	II	HCV	positive	negative	CsA
Fourth	II	No	negative	negative	CsA
Fifth	II	HCV	negative	negative	CsA
Sixth	II	No	negative	negative	Tacrolimus

4. Discussion

The aim of this work was to detect reactivation of CMV in renal transplant recipients and to compare CMV serology, the method routinely used, with qRT-PCR which is an important tool in predicting subsequent or ongoing disease. In addition, our aim was to determine those who are more prone for CMV reactivation and thus may require more frequent CMV monitoring and to determine which postoperative period for CMV reactivation most likely to occur.

Our results showed that as regards the pre-transplantation CMV serostatus; all recipients (100%) as well as all donors (100%) had positive anti-CMV IgG and negative anti-CMV IgM, indicating that all of them were infected with CMV before transplantation**.** The high prevalence of CMV seropositivityobserved in this study was in agreement with the previous studies [(8-10)].

The importance of CMV in a renal transplant population is the interplay between the background frequency of infection in the donor and recipient population and the intensity of immunosuppression [(11)]. The lack of specific immunity in the recipient allows a significant replication of CMV, resulting in symptomatic infection (CMV disease) that is sometimes highly severe. While in case of reactivation, both humoral and cellular immunity of the recipient decreases virus replication dynamics, therefore reducing disease incidence and severity [(4)].

In the present study regarding the CMV serostatus of the donors and the recipients, they belonged to the D+/R+ group which has intermediate risk for reactivation of CMV.

As regards the timing of CMV reactivation post transplantation the results of this study showed that 2 patients had CMV reactivation by real-time PCR. CMV reactivation was observed in group II i.e. during the 2nd to 6th month postoperatively (10%) as detected by positive PCR while in group I and III no CMV reactivation had occurred. This result was in accordance with[(12-17)],who reported that The second to the sixth month post transplantation is the period where opportunistic infections such as CMVaremost common as a consequence of the intensity of immunosuppressive therapy in that period.

The results of our study showed that CMV reactivation occurred in two patients following the treatment of their rejection episode Thus, there was a high percentage of CMV reactivation following treatment of an episode of acute allograft rejection. These results were in agreement with Kamaret al.[(18)],who found that treatment of an episode of acute allograft rejection increased the risk forCMV reactivation within the first year post-transplant by more than eight folds, this might be related either to the high doses of methylprednisolone or cytolytic lymphocyte-depleting therapy. Therefore, they recommended that in CMV-seropositive patients, when treatment of acute rejection is attempted, CMV

prophylaxis should be implemented if it is not already the case [17-19].

Previous studies showed a significant correlation between CMV disease and acute rejection episodesand reported that this was due to the cumulative immunosuppressive effects during the anti-rejection therapy which increase the risk of CMV infection. This indicates that anti-rejection treatment is an independent risk factor for CMV disease [20].

Patients receiving antilymphocyte antibodies, or antithymocyte polyclonal antibodies or monoclonal antibodies OKT3, are considered at high-risk for CMV reactivation. These preparations contain cytotoxic antibodies to antigens expressed in human lymphocytes, induce T cell depletion and release of cytokines, mostly tumour necrosis factor α, leading to reactivation of infections caused by Herpesviruses (mainly CMV and EBV)[4].

Out of the fifty-two patients who received cyclosporine (CsA), as a calcineurin inhibition in their immunosuppressive regimen; two patients had positive CMV DNA by PCR. These results came in contrast to Ekberg et al.who found that the incidence of CMV infection was higher in kidney-transplant patients treated by standard doses of cyclosporine (14%) compared to those receiving tacrolimus (10%). (21). In addition, the results of the present study were contradictory to the study by Chakravartet al. (2009) who reported a four-fold increase in the incidence of CMV disease in renal transplant recipients after introduction of cyclosporine immunosuppression [22].

As regards the post transplantation CMV serology in the present study, all patients were screened for CMV IgG and IgM post operatively by ELISA test system. Anti -CMV IgG was positive in all patients (100%) while Anti-CMV IgM was positive in only 3 patients (5%). These 3 patients have their CMV PCR negative and they did not suffer from any post transplantation rejection episode during the period of the study.

One possible explanation for this observation is the persistence of CMV-IgM antibodies in serum, specifically antibodies against pp150 tegument after a recent CMV infection. It has been shown that, in the same patient group, serum CMV-IgM concentration is detectable for months or even years after infection. Long-term persistence of these antibodies would preclude any crucial diagnostic role for them, because positivetests after conversion cannot be properly interpreted[23].

In the present study, 2 patients were CMV realtime PCR positive and simultaneously negative for anti CMV IgM. This may be explained by the failure to detect anti-CMV IgM during the 6 to 8 week window phase immediately after infection. Importantly, these individuals can have high peripheral-blood viral loads, suggesting that their blood would be potentially infectious[24].

This coincides with previous studies [23,25,26], who reported that serological assays lack the usefulness in diagnosing CMV reactivation in transplant patients and they have only confirmatory value after transplantation. This is because in some cases antibodies may not develop due to immunosuppression, may develop after the disease is already cured, or may persist for years[23]. Only ELISA can be used as a screening index in order to distinguish whether the donors or recipients are infected with CMV or not, but much lessso for determining viral responses maybe blunted[27].

In the present study, 19 patients (31.66%) were co- infected with HCV, 2 of them (2/19 i.e. 10.5%) had CMV reactivation detected by real-time PCR.

Hepatic dysfunction after kidney transplantation is expected to be more prevalent among Egyptians due to the impact of schistosomiasis and HCV.

Thus, the result of this study was in agreement with previous studies,it was suggestedthat HCV-RNA might contribute toCMV reactivation in HCVseropositiverenal transplant recipients [28, 29].

In the present study one of the two patients with positive CMV real-time PCR had his ESRD due to diabetic nephropathy; this finding may support the previous finding by Yangoet al.[30]. This observation could be due to the suppressive effect of DM on the immune system, which increases the risk of developing CMV disease[30].

In this study, CMV DNA detection and quantification was done using real-time PCR assay first on whole blood then on plasma; where it was detected in whole blood but not in plasma in two patients belonging to group *II*. These two patients did not experience any symptoms of CMV disease during the time of performing the QnPCR. In the present study, CMV DNA was detected by in whole blood but not in plasma. These results are in agreement with the report of Razonable and Emery[31]. They recommended the use of whole blood for the polymerase chain reaction (PCR) in the diagnosis of CMV infection and the use of CMV load measurements for prognostication and for monitoring response to anti-CMV therapy. Higher viral loads are detected in WB than in plasma; since CMV replication starts in cells and is followed by the release of viral particles into plasma .Thus, CMV monitoring in whole blood could be superior to assays in blood cells or plasma alone [32].

5. Conclusion:

Cytomegalovirusreactivation was not high in the studied patients, which may be due to the presence of

pre -existing immunity in the form of neutralizing antibody.Reactivation of CMV occurred more frequently during the 2nd to 6th month postoperatively and that treatment of an episode of acute allograft rejection increased the risk for CMV reactivation within the first year post-transplantation.

Whole blood (WB)real-time quantitative PCR seems to be an appropriate candidate for routine performance, to monitor CMV infection in transplant patients. IgM seroconversion was not predictive of subsequent CMV disease and that ELISA can only be used as a screening index in order to distinguish whether the donors or recipients are infected with CMV or not, but not to determine viral reactivation.

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