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RESEARCH ARTICLE

BK polyomavirus and Cytomegalovirus Co-infections in renal transplant recipients: a single center study

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Abstract

Background: Opportunistic viral infections make an important threat to renal transplantation recipients (RTRs), and with the use of more intense newly-developed immunosuppressive drugs; the risk of renal allograft loss due to reactivation of these viruses has increased considerably. At the top priority of these viruses lie BK polyomavirus (BKV) and human cytomegalovirus (CMV). Reactivation of these viruses in these chronically immunosuppressed RTRs can lead to renal impairment and subsequently allograft loss, unless early detected and properly treated.

Objectives: The study aimed to detect and quantify plasma viral load of BKV and CMV in RTRs using quantitative real time PCR (qRT-PCR), in order to study the prevalence of these two viruses in the sole renal transplantation center in Baghdad, and correlate viral load with the diseases severity. Furthermore, the prevalence of BKV-CMV coexistence in RTRs, to find out whether infection by one of them is a risk factor for infection by the other was investigated.

Patients and Methods:A total of 99 RTR were enrolled in the study, and 15 non-transplanted patients with chronic kidney diseases (CKD) together with 15 health living donors (LD) were taken as controls. Plasma samples were taken from all participants. From which viral DNA was extracted, and then real time PCR technique was used to measure the viral load.

Results: Out of 99, 12 (12.12%) of RTR patients were positive for BK viremia with a viral load (VL) ranging from ($1x10^2$ to $1x10^9$ copies/ml), while none of the control groups was BK positive, and 5 patients out of these 12 had BKV nephropathy. For CMV, 13.13% of RTR patients had positive CMV viremia with a VL ranging from ($1.25x10^2$ to $7.94x10^7$ copies/ml), and only one of the CKD controls was CMV positive. Only 3 patients had BK-CMV coexistence, which was statistically not a significant risk factor for one another.

Conclusion: Our study suggests that both BK polyomavirus and CMV should be considered important causes for nephropathy and allograft loss in RTRs in Iraq.

INTRODUCTION

With the development of organ transplantation, kidney transplantation has become very effective therapeutic option for ESRD (end-stage renal diseases). Simultaneously, this has resulted in a series of challenges, mainly donor deficiency, expense, and transplant-related complications like rejection, and opportunistic viral infections (1,2).

Opportunistic polyomaviruses infections mainly BK virus (BKV) and JC virus has become increasingly common problem. Polyomaviruses are circular, double-stranded DNA viruses (3). The most important and commonest among these viruses is BKVinfection, which was reported in ~15% of renal transplant recipients (RTRs) in the first post-transplant year in the absence of an effective prophylaxis strategy (4,5).BKV presents with an asymptomatic gradual rise in serum creatinine with a tubulo-interstitial nephritis mimicking rejection, making a treatment dilemma. Reduction in immunosuppression that is needed to treat BKV infection is opposite to their importance to overcome allograft rejection (6).

After a primary infection, BKV establishes its latency in the urinary epithelium and renal tubular epithelial cells. When starting immunosuppression, the virus is reactivated and begins to replicate, causing BK viremia and eventually invades the renal allograft, leading to BK virus nephropathy (BKVN). The rates of BKVN varies, ranging from 1 to 10% (4,5,7,8).

The second opportunistic pathogen is human cytomegalovirus (CMV), which is a major cause of morbidity in renal transplant recipients. CMV seropositivity is common in the general population, with a prevalence ranging from 30 to 97%. After the primary infection, CMV establishes a life-long latency in various organs (9-11). In RTRs, CMV disease is associated not only with considerable morbidity and mortality, but also with increased risk of acute rejection, interstitial fibrosis and tubular atrophy or chronic rejection, and finally impaired long-term graft and patient survival (12-15).

Co-infection of BKV and CMV has been reported in RTR by several studies (16-20), and CMV has been shown to induce polyomaviruses amplification and DNA replication in vitro (21-22).In Iraq active kidney transplantation program was started in 1973, and since then, renal transplantation is being done in many centers in Iraq (23-25). However, to the best of our knowledge, there is no previous study which deals withthe prevalence of BK polyomavirus, and CMV-BK co-infections in Iraqi RTRs, therefore, this study aimed to investigate the prevalence of BKV and CMV inIraqi RTRsusing quantitative real time PCR (qRT-PCR).

Subjects and Methods

Patients

This cross-sectional study was conducted from March 2013 to March 2014. A total of 99 RTR patients who attend the (Center of Kidney Diseases and Transplantation) in the Medical City of Baghdad, were enrolled in the study. A consent letter was signed by each patient, and the study was approved by the ethical committees of the Ministry of Health and Al-Nahrain University. Plasma and urine samples were collected from the patients, 33 of them had normal renal function, and the remaining 66 had impaired renal function, 78 were males. Two control groups were included in the study, 15 Living Donors (LD) and 15 non-transplanted patients with Chronic Kidney Disease (CKD).

Methods

DNA was extracted from 200µl of plasma using a Bosphore® Viral DNA Extraction Spin Kit according to the manufacturer's protocol which is based on the silica membrane column separation method (Anatolia Geneworks, Turkey) and was eluted from the column with 40µl elution buffer.

Quantification of BKV and CMV plasma viral load was done Usin**g**Bosphore® BKV/CMV Quantification Kits (respectively) which detect and quantitatethe four main genotypes of BK Virus DNA in human plasma. A region within the BKV large T-antigene (LT) encoding gene is amplified. While CMV quantification kit, can detect and quantitate human CMV DNA in plasma, and encompassing all major CMV genotypes. A region within the CMV DNA polymerase gene is amplified. Fluorescence detection is accomplished using the FAM filter (for both viruses), and to check PCR inhibition an internal control is incorporated into the system. The internal control was detected with the Cy5 filter. Quantitation of BK viral load was performed using four quantitated BKV DNA controls, ranging from $1x10^4$copies/ml to $1x10^7$copies/ml. And quantitation of CMV viral load was done using internationally accepted DNA quantitation standards ranging from $5x10^2$copies/ml to $5x10^5$copies/ml.

Statistical analysis

Statistical analysis was performed with the software SPSS version 21.0, and Microsoft Excel 2013. Categorical data formulated as count and percentage. Fisher exact test was used to describe the association of these data, in addition to relative risk study (RR). Numerical data were described as mean, standard deviation of mean.

Independent sample t-test used for comparison between two groups while analysis of variance (ANOVA) was used for comparison among more than two groups. $p \leq 0.05$ was considered statistically significant.

Results:

Out of 99 RTR, 78 (78.79%) were males. Their mean age was 37.01±1.31 ranging between 18 and 67 years, the mean ages of the two control groups were (38.5±2.8) and (38.4±2.7) years for LD and CKD respectively. The mean serum creatinine value in the RTR was 2.33±1.7, and their mean post-transplantation period was 17.53±0.97 months ranging from 2-30 months.Among these 99 RTRs, 19.19% had renal allograft rejection, five of them (5.05%) were receiving antithymocyte globulin (ATG) as anti-rejection therapy, 5.05% had biopsy proven BK virus nephropathy (BKVN), 4.04% had ureteric stenosis, and 43.44% had donor+/recipient+ CMV serostate, figure (1).

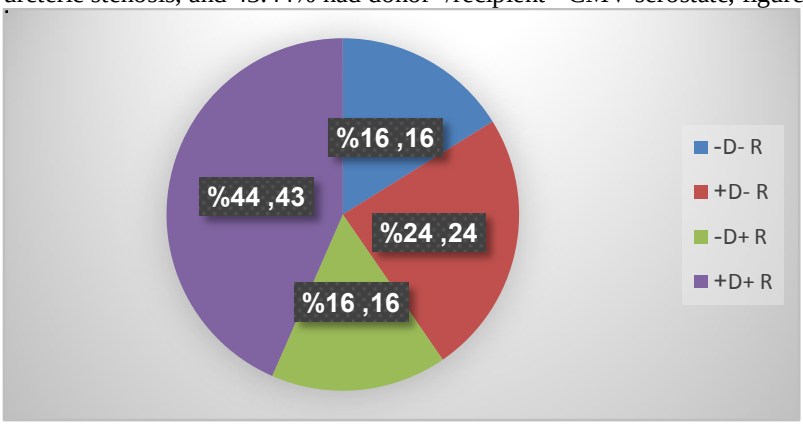

Figure (1): Distribution pattern of (D) Donor/ (R) Recipient CMV serostate in the 99 RTRs

Two main standard immunosuppressive regimes are mainly followed in our transplantation center in Baghdad; the old regimen which includes cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, the second regimen includes tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone, figure (2).

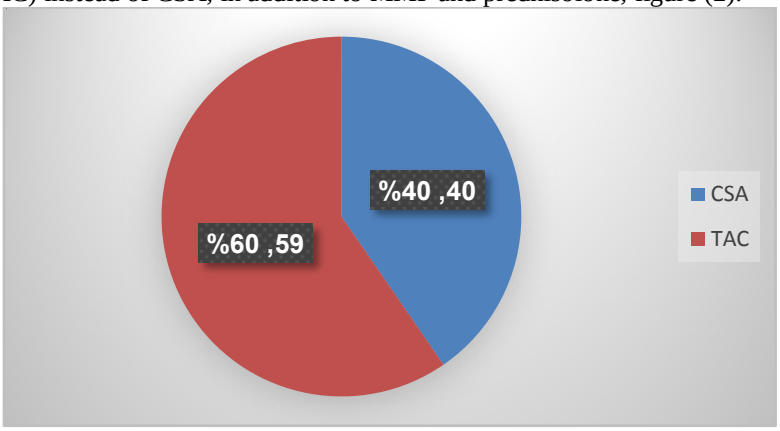

Figure (2): Distribution pattern in the use of the two immunosuppressive regimens in the 99 RTRs

BK viremia was detected in (12.12%) 12 out of 99 RTR with a viral load (VL) ranging from $1x10^2$ to $1x10^9$ copies/ml, and none of the control groups (LD and CKD) was BKV positive. Among the 12 BK positive cases, 7 patients were above 40 years old and 6 of these 7 patients had BK VL more than 10^3copies/ml, 10 out of 12 were males and 7 out of these 10 males had BK VL more than 10^3 copies/ml, 9 out of 12 had renal transplantation within less than 12 months and 6 of these 9 patients had BK VL more than 10^3copies/ml,

According to our standard curve,58.3% (7/12) had BK viral load more than $1X10^3$copies/ml, with a mean of $1.99X10^8$copies/ml. In addition, 41.7 % (5/12) had biopsy proven BKVN, i.e. 5.05% (5/99 RTR) had BKVN, 4 of these 5 cases (80%) had BK viral load of more than $1X10^4$ copies/ml, and three of these 5 cases (60%) have lost their graft.

Regarding donor/ recipient CMV serostate, there was no significant difference in donor/ recipient CMV serostate among the 12 BK viremic patients. However, the study revealed a significant positive correlation between serum creatinine value and BK VL; (r=+0.576, p=0.05), figure (3). But, only 3 out of the 19 RTR patients who had rejection, had BK viremia; which shows no significant correlation (p=0.581), and only one of the 5 patients who were receiving ATG (anti-thymocyteglobuline) had BK viremia. There was a significant difference in BKV viremia rates between samples from the biopsy proven BKVN and remaining BK positive cases (p=0.03). Furthermore, the study showed a highly significant association between BKV positivity and ureteric stenosis cases (p=0.007).

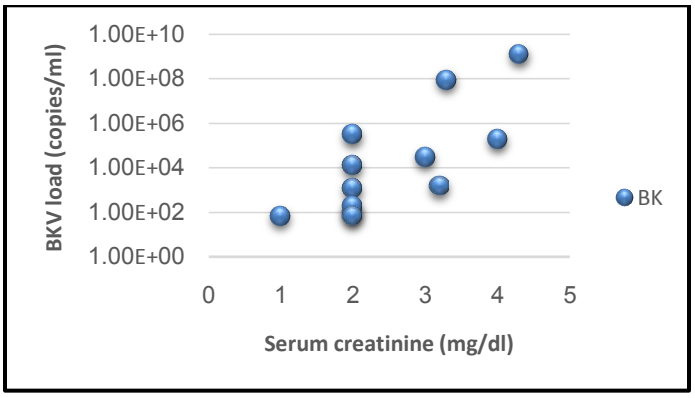

Figure (3): Correlation between serum creatinine value and BK viral load (r=+0.576, p=0.05)

Despite the none significant correlation between the type of immunosuppressive regimen used and BK viremia (p=0.42), this study showed that 50% (4 out of 8) BKV positive patients who were receiving TAC regimen had BK VL more than $1x10^4$ copies/ml, and all of the 5 patients who had biopsy proven BKV nephropathy (BKVN) were on TAC regimen.

CMV viremia was detected in (13.13%) 13 out of 99 RTR with a median viral load (VL) of ($2.69x10^3$ copies/ml), ranging from ($1.25x10^2$ to $7.94x10^7$ copies/ml), while only one of the CKD controls had positive CMV viremia, and none of the LD group had CMV viremia. Table (1) demonstrates the clinical presentations of symptomatic CMV viremic patients, in which 8/13 (61.5%) of CMV viremic patients had CMV disease with viral load more than $1x10^3$copies/ml.

Table (1): Clinical presentations of the symptomatic CMV viremic RTR patients

Diseases	Pneumonia + fever	CNS disease + fever	Renal Allograft Rejection	Died
No. of cases	3 (23%)	1 (7.7%)	2 (15.4%)	2 (15.4%)

This study showed a highly significant difference in D/R CMV serostate among the 13 CMV positive patients (P=0.005), in which 12 out of these 13 patients had donor positive CMV serostate. In addition, the study found a significant difference in serum creatinine value between CMV viremic and non viremic patients (p=0.014).However, there was no significant correlation between CMV viremia and age, gender or duration of transplantation. In addition, only two patients who had CMV viremia had renal allograft rejection, which is not significantly correlated with rejection in our patients (p=0.712), and none of the 5 RTR patients who were receiving ATG as anti-rejection therapy had CMV viremia. Finally, this study showed no significant difference in the type of immunosuppressive regimen used and CMV viremia state (p=0.223).

The results of our study demonstrated that only three out of the 12 BK and 13 CMV viremic patients had combined BK-CMV infections. Table (2) demonstrates the demographic data of these three patients, in addition to BK and CMV viral loads and clinical presentations of them. Relative risk study showed that neither BK infection nor CMV is a risk factor for the other, table (3).

Table (2): Renal transplant recipients who had BK and CMV co-infection

Case no.	Age/y	Gender	PTP/ month	S. Cr mg/dl	BKV Copies/ml	CMV Copies/m	D/ R	Immunosuppressive regimen	Clinical diseases

						l			
1	25	M	6	2	3.09 X10^5	1.90 X 10^3	+/+	MMF, PRD, TAC	Ureteric stenosis, BKVN & rejection
2	51	M	14	2	3 X 10^4	5.37 X 10^2	+/-	MMF, PRD, TAC	BKVN
3	18	M	7	4	1.82 X 10^4	8.91 X 10^3	+/+	MMF, PRD, TAC	Fever &meningoencephalitis with impaired consciousness. Died

Table (3): Relative risk (RR) study for BK-CMV co-infection

	RR	95% CI	z statistic	P-value
For BKV	2.175	0.695-6.803	1.336	0.182
For CMV	2.205	0.685-7.099	1.326	0.185

Discussion

With the use of more potent immunosuppressive regimens to decrease acute rejection rates in RTRs, viral infections had emerged as an important cause of allograft loss. BKV is one of the common post-transplant viral infections (4,7), in this study, the prevalence of BK viremia was 12.12%, which lies within the range of overall incidence of BK viremia that range from 11% to 29% (26,27). Following the detection of viruria, some patients develop viremia. BK viremia is believed to result from a more extensive infection leading to severe tubular injury with rupture of tubular basement membranes and entry of the virus into the blood stream via peritubular capillaries. Ultimately, sustained viremia is associated with BKVN in 1% to 10% of RTRs (28-30), in which also the prevalence of BKVN (5.05%) in our study lies within this range.

This makes us to raise a question: Why 1-10% developed BKVN and others didn't? The answer lies mainly in two main categories (viral genetic alterations and patient immune status), imbalance between BKV replication and BKV-specific immune control are viewed as a key element of pathogenesis (31). Genetic alterations in VP-1 may be important with regards to (a) modulation of tissue injury, (b) evasion of host neutralizing antibodies, and (c) escape from VP-1 specific T-cell responses (32). In 2010, Tremolada et al. suggested that rare BKV VP1 variants are more frequently associated with disease and some variants were more cytopathic than others in RTRs (3).

Studies found that BK viremia and BKVN are most common in the first year after transplantation, when immunosuppression is most intense (16,33), which are in line with our results which showed that (75%) 9 out of 12 had renal transplantation within less than 12 months, and 66.7%, 6 of these 9 patients had BK VL more than 10^3copies/ml. However, some workers found that early, during the first month following transplantation, BKVN can occur only exceptionally (8). This explains our reason behind collecting RTR patients with a post-transplant period ranging between 2-30 months and not less than 2 months.

In addition, studies showed that BKV reactivation is usually associated with ureteric stenosis and bacterial urinary tract infections (16,33), which also support the results of this study in which 2 out of the 12 viremic patients had bacterial urinary tract infection, and another 2 out of the 12 viremic patients had ureteric stenosis (among the only 4 out of 99 RTR who had ureteric stenosis) (p=0.007).

In the majority of patients, BK viremia and progression of BKVN are asymptomatic except only for steadily increasing serum creatinine concentrations (31,34,35). A presentation that is very obvious in our viremic patients is that there was a significant positive correlation between serum creatinine value and BK VL; (r=+0.576, p=0.05), figure (2). And this increasing serum creatinine often makes most of the physicians to misdiagnose BKVN as an acute rejection or drug toxicity (36).

Results of our study showed a significant difference between the median BK VL in plasma from the biopsy proven BKVN and the remaining BK viremic patients ($1.82x10^5$ versus $1.82x10^2$) copies/ml (p=0.03), in which 4 out of 5 BKVN cases had BK VL levels more than $1x10^4$ copies/ml. The American Society of Transplantation (AST) defines a BK VL of ≥4 log10/mL ($1x10^4$copies) as presumptive BKVN, and recommends reduction in immunosuppression (31), which is also supported by other studies (28), which is in agreement with our results.

However, a recent study showed that these current AST guidelines have underestimated the diagnosis of BKVN, and a cut off BK VL of ≥ ($1x10^4$ copies) might not necessarily indicate a presumptive BKVN, and even lower values of

plasma viral load could sometimes associated with BKVN (37). This finding explains why one of the 5 BKVN cases in our study had BK VL less than ($1x10^4$ copies).

The suggested risk factors for BKVN and BK viremia, included mainly the recipient factors (older age, male gender, low or absent BKV-specific T-cell activity) (31,38), in the present study, although it was not significant by relative risk study, 7 patients out of the 12 BK positive cases were above 40 years old and (85.7%) i.e. 6 of these 7 patients had BK VL more than 10^3 copies/ml. Also (83.3%) 10 out of 12 were males and (70%) 7 out of these 10 males had BK VL more than 10^3 copies/ml.

Although RR study showed none significant correlation between the type of immunosuppressive regimen (whether TAC or CSA) and BK viremia, 66.7% (8/12) patients were on TAC regimen, 50% (4/8) had BK VL more than $1x10^4$ copies/ml, and all of the 5 patients who had biopsy proven BKV nephropathy (BKVN) were on TAC regimen, a finding that is in agreement with the study of Hirsch et al(5), which showed that high-titer BK VL (>4 log) 10^4copies/ml, and the overall median BK VLs were higher in the TAC group.

In 2009, Egli et al. (39) demonstrated that TAC therapy inhibits BKV-specific T cell immune response, and the reduction of immunosuppressive therapies has led to an increase in the BKV-specific cellular immune response. However, both TAC and CSA were shown to cause dose-dependent inhibition of IFN-γ expression by BKV-specific T cell response (6,40).

Among the most common infectious complication in patients with solid organ transplantation is CMV infection, despite the major advances in CMV disease prevention in the past decade (41). Using antiviral prophylaxis or preemptive therapy has resulted in a decrease in the incidence of CMV disease from 20-60% to 5-20% (42), and our study results that showed a rate of 13.13% (13/99) CMV reactivation and replication are falling within this range.

The manifestations of CMV diseases range from mild febrile illness to tissue-invasive disease,like gastrointestinal disease, pneumonitis, chorioretinitis or meningoencephalitis (41). One patient in this study had meningoencephalitis with impaired consciousness. The study of Helanter et al (43), found that one of the most common symptoms of CMV infection is fever, as in the current study which showed 50% (4 out of the 8 symptomatic patients) had fever as a primary presenting symptom.CMV disease is also associated with increased risk of acute or chronic rejection, interstitial fibrosis, tubular atrophy and finally impaired long-term graft and patient survival (12-15), which support the results of our study that state a 100% (13/13) of CMV positive RTRs had impaired renal function with a mean serum creatinine of 2.7±1.7 mg/dl (p=0.014), and two of them had chronic rejection.

The results of our study are in agreement with the results of Boudreault et al (44), and Helanter et al (43), who studied the risk factors of late-onset CMV disease among RTRs and showed no significant correlation between CMV infection and age, sex, and type of maintenance immunosuppression in RTRs, or even the use of antirejection therapy like ATG.

CMV seronegative recipients of an organ from a seropositive donor (D+/R–) are at greatest risk of CMV infection. Without prophylaxis up to 50% of these high-risk patients will develop symptomatic infection (45,46), which support the results of our study in which the prevalence of CMV viremia was 37.5% and 14% in D+/R- and D+/R+ RTRs respectively (p=0.005). And lowest in D-/R- RTRs 6.25% which is in agreement with other previous studies (46,47).

Several studies focused on the association between BKV and CMV infections both in RTRs (16-20), and in hematopoetic stem cell transplant recipients(HSCT) (48-50). Some of these studies showed that plasma BK-positivity is highly associated with co-infection of CMV, suggesting a possible risk factor for one another. Therefore, detection of either infection strongly suggests the need to investigate for the other (17,18).In contrast, the results of this study showed no significant association between these two viruses in RTRs using relative risk study, a result which in accordance with that of Nasiri et al (19), though their patients were all taken at one month post-transplantation, which is different from our patients who were taken after two months of transplantation operation.

However, the current study found that 2 out of these 3 (BKV-CMV) co-infected patients had biopsy proven BKVN, and the third patient had CMV-induced meningoencephalitis with impaired consciousnessand died within 10 days. Furthermore, all of these three patients had BK VL more than $1x10^4$ copies/ml, indicating that detection of one of these two viruses could justify monitoring for the other, in spite of the statistically none significant association.

The interaction of CMV and BKV has been investigated in vitro, as both viruses might be reactivated by immunosuppression or even, other states of host-virus imbalance. Interestingly, CMV has been shown to induce polyomaviruses amplification and DNA replication in vitro (21,22). BKV large T antigen was found to be able to enhance the expression of immediate early (IE) and early (E) CMV genes (51).

On the other hand, Heilbronn et al (50) have shown that CMV induces JC virus DNA replication, and that ganciclovir-induced inhibition of CMV replication was associated with a concomitant inhibition of JC virus replication. The temporal association between BK virus-associated hemorrhagic cystitis and reactivation of CMV in the study of Bielorai et al (48) that had two HSCT patients with BKV-CMV co-infections led them to hypothesize

that as in the case of JC virus, CMV was responsible for the replication of the BK virus and hence for the resultant hemorrhagic cystitis. Treatment with ganciclovir controlled the CMV infection, and as a consequence of inhibition of the BK virus replication resolution of the hemorrhagic cystitis symptoms occurred (48).

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