Original Article

A Study on BK Polyomavirus among Kidney Transplant Recipients and Nontransplants

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Abstract

Background: BK polyomavirus (BKV) induces allograft malfunction in renal transplant recipients (RTRs) and it could cause loss of the allograft, however, this virus does not cause any harm among healthy subjects. **Aims:** This study was conducted to determine and compare the frequency of BK viremia between RTR and healthy subjects, and to find out its risks and its relation to their renal function. **Settings and Design:** This was a case–control study. **Subjects and Methods:** A total of 206 blood samples were collected from (106) RTRs within the first 2 years posttransplantation from the center of kidney diseases and transplantation, and (100) nonrenal transplant samples (healthy blood donors from the Iraqi Blood Donation Center in the Medical City of Baghdad. The large tumor antigen region of BKV was amplified by a real-time polymerase chain reaction. **Statistical Analysis Used:** Frequencies, percentages, Chi-square-test, odds ratio (OR), and confidence interval were used for statistical analysis by SPSS v. 28 (IBM ,USA). **Results:** BKV was positive in 23 (21.7%) of RTR patients and 8 (8.0%) of control, which is statistically significant $P = 0.005$. RTR patients under tacrolimus (TAC) were at a higher risk, to had BKV viremia ($P = 0.05$). However, there was no significant difference neither in relative risk (OR = 0.904) nor the distributions ($P = 0.839$) regarding serum creatinine levels. **Conclusions:** A significantly higher BK viremia among RTR and increasing risk of reactivation with TAC immunosuppression should warn the nephrologists about the risk of this immunosuppression regimen on the renal allograft.

Keywords: BK polyomavirus, immunosuppressive drugs, real-time polymerase chain reaction, renal transplant recipient

Introduction

Infections with the BK polyomavirus (BKV) are most commonly transmitted during childhood, usually through oral or nasal transmission. Infections with the BKV are primarily acquired early in life, most often through oral or respiratory transmission.[1] It can replicate to harmful levels in immunosuppressed persons even though it creates a persistent asymptomatic infection in urinary epithelial cells. In up to 10% of kidney transplant recipients (KTRs), BKV produces nephropathy BKV Nephropathy (BKVN). Researchers have shown a correlation between BKV genotypes Ib2 and IV and BKVN. It appears that the virus in the donor's kidney can "travel" and proliferate at high levels in recipients who initially lack neutralizing antibody responses to BKV genotypes present in the graft.[2] Renal transplant recipients (RTRs'), BK viremia rate, and renal function were the primary goals of this study.

If an RTR has positive BK viremia, then study the relationship between this positive BK viremia and the patient's serum creatinine. The infection of transplant patients with BKV has resulted in serious consequence that affects the recipients' renal function. In renal transplant patients, BKV infection has been linked to polyomavirus-associated nephropathy and allograft loss.[1-4] It is controversial if BKV infection causes a deterioration in renal function in liver transplants. Loeches *et al*. discovered that persistent BKV viremia might be related with lower renal function in liver transplants in a prospective study.[5,6]

Other studies, however, found no link between BKV viremia and viruria and renal impairment in liver transplant recipients.

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The difference between the strong association of BKV infection with renal allograft function in RTRs and the lack of a causal relationship between BKV infection and renal function in liver transplant recipients may underline the importance of a second hit, such as ischemia or inflammation, in the development of renal dysfunction in addition to BKV infection.[7,8]

This study was conducted to determine and compare the frequency of BK viremia between RTR and healthy subjects, and to find out its risks and its relation to their renal function.

Subjects and Methods

Patients

This case–control study was carried out between November 2021 and March 2022. Samples were collected at Baghdad's Medical City from the Center of Kidney Diseases and Transplantation and the Iraqi Blood Donation Center after the approval of the ethical committee and patients' consent.

This study was conducted in the Medical Research Unit of Al-Nahrain University's College of Medicine. A total of 206 plasma samples were collected from (106) RTRs within the first 2 years after transplantation, and (100) healthy blood donors.

Methods

A total of 206 plasma samples were collected from 106 RTR patients and 100 healthy blood donors and subjected for viral DNA extraction from100lµl of plasma using a WizPrep™ Viral DNA/RNA Mini Kit (V2) according to the manufacturer's protocol, which is based on the silica membrane column separation method (Wizbiosolutions, Korea), and 40 µ was eluted from the column from elution buffer. A region inside the BKV large tumor-antigen (LTA) encoding gene is amplified using real-time polymerase chain reaction (RT-PCR). For quantitative real-time, the Qiagen RT-PCR system (Corbett RotGeneene Technologies, USA) with qPCR soft software was utilized. Viral detection was quantified by measuring the cycle threshold (Ct) using the 2EasyTaq qPCR Master Mix Kit components. Negative controls included a nontemplate control, a nonamplification control, and a nonprimer control. Each reaction was done in duplicate non primer control (NPC). TaqMan fluorescent oligonucleotide probes and primers sequences were designed in this study, synthesized by Alpha DNA Ltd (Canada), and stored lyophilized at −23°C. The sequences of each of the probes and primers used in detection virus experiments are shown in Table 1, and Table 2 shows the thermal profile of RT-PCR.

Results

The RT-PCR run was carried out for detection of BKV in plasma, and out of the total 206 subjects, 31 (15.0%) were BKV positive, the mean BKV Ct value was 20.99 ± 2.737.

The number and percentage of studied groups according to the positivity of BKV DNA by RT-PCR were as follows: 23 (21.7%) of RTR patients, while 8 (8.0%) from control were positive, which is a statistically significant $P = 0.005$ [Figure 1].

Table 1: Primers and probes of this study

Primer/probe	Sequence (5′→3′ direction)
BK polyomavirus was detected	
Forward	CATTTTATCCTCGTCGCCCC
Reverse	AAAGAGCTGCCTGGGGAAAT
FAM - probe	TGTCAGGGTGAAATTCCTTACAC
BK polyomavirus sequence 346 bp	
Forward	CTTGCCTGCTTTGCTGTGTA
Reverse	TCCAAGACACCTGCTTTGTT

Table 2: Real-time polymerase chain reaction thermal profile for the detection of BK polyomaviruses

Step	Temperature (°C)	Duration (s)	Cycles
Enzyme activation	94	30	Hold
Denature	94	5	
Annealing	58	25*	40
extension	72	20	

*it is for duration by seconds

However, there was no statistically significant difference in the distribution of BKV DNA positivity regarding the PTP ($P = 0.371$), as in Table 3, which showed 18 (24.0%) from RTR who were positive BKV DNA have PTP equal to or <12 months and 5 (16.1%) were more than 12 months. While according to the type of immunosuppression regimen, there was a significant difference in the BK viremia as shown in Table 4, whereas 19/23 (82.6%) BKV viremic patients were on tacrolimus (TAC) regimen, and the remaining 4/23 (17.4%) were on cyclosporine (CYC) regimen ($P = 0.05$).

When the relative risk was analyzed, the results shows that the patients under TAC chemotherapy were at a higher risk, to had viremia of BKV, than those whom under CYC chemotherapy with significant differences statistically (odds ratio [OR]: 1.344 $P = 0.05$). To evaluate the impact and the relative risk of BKV positivity on renal function, which is represented by serum creatinine, the results showed no significant differences neither in relative risk (OR: 0.904) nor in the distributions [$P = 0.839$, Table 5].

Discussion

BK virus which is the etiological agent for BKVN, currently, there is no specific treatment for BKVN except for reducing immunosuppression, risking the reoccurrence of rejection. PCR was used only in a very small number of studies to investigate the identification of viral infections or reactivations in Iraqi kidney transplant recipients.[9] The detection of BKV in the blood, which can be quantified by measuring the Ct, is a valuable tool not only for BKV nephropathy diagnosis but also for monitoring the patient's response to treatment. However, regardless of the amount of virus present, RT-PCR is a very sensitive and specific approach for identifying BKV in blood components (plasma or serum).[10] In these kinds of situations,

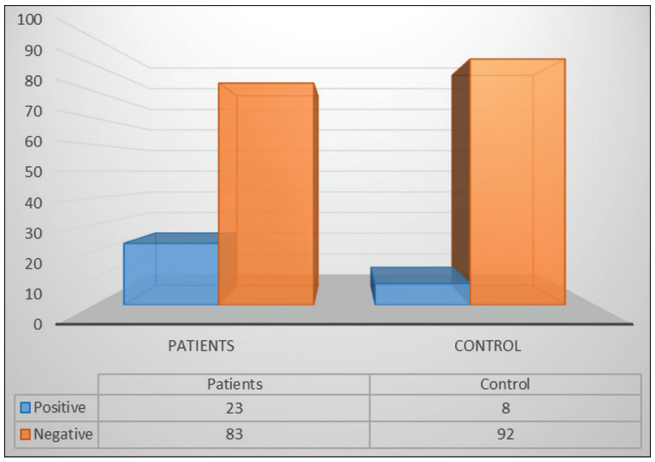

Figure 1: Distribution of positive BKV DNA in each study groups. BKV: BK Polyomavirus

the sensitivity is 100%, however, the specificity is just around 90%, this is due to the fact that the restriction of the immune system allowed latent viruses to resurface, replicate in blood, and resume their replication, causing damages in renal tubular cells comparable to those caused by rejection. Utilizing an RT-PCR kit that can identify moderately low levels of viral antigen is critical for screening tests.[11]

The prevalence of BKV load was more elevated in the 1st year compared with the 2nd-year posttransplantation follow-up visits.[9] In the current study, the results on the finding of BKV DNA from plasma samples examined of the total 206 (100%) of both 106 RTRs patients and 100 apparently healthy controls group, 31 (15.0%) were positive BK viremia, this falls about in the middle of the overall incidence of BK viremia, which may be anywhere from 11% to 29%.[12]

Conclusions

This conclusion is consistent with the findings of Al-Obaidi *et al*., 2015, in which BK viremia was shown to be present (12.12%) in 12 out of 99 Iraqi RTRs. BK viremia is considered to be the outcome of a more widespread infection that led to significant tubular damage. Once the tubular basement membranes burst and the virus enters the circulation through the blood capillaries.[13] However, there was controversy in the results of two groups of local research articles. The first group that of Mohammad, 2016 reported a very low percentage of BK viremia was 2/72 (2.8%), and the second group showed a high percentage like our finding (11/50 (22%) in Iraqi.[10,14] In contrast to our finding, where there was a positive result of RT-PCR for BKV-DNA in the control group (8.0%), several local researchers reported no detection of BKV_DNA in their control group such as Al-Obaidi *et al*., 2015, and Shamran *et al*., 2016. The analytical sensitivity of the kit utilized for the viral DNA quantification will be responsible for this variation. The outcome can also be influenced by the target gene. However, Shamran *et al*. utilized a kit that targets small T-antigen, whereas the kit which was used in this study and Al-Obaidi *et al*. employed targets the LTA gene. In a global cohort examination of patients with Greek RTRs, the incidence of BK viremia was as high as 31%.[10,15]

In general, variations in sample type, size, DNA extraction procedures, primers, probe sequences, and BKV strain DNA used to generate the standard curve might influence quantification findings and introduce clinically meaningful variability. The low prevalence of BKV in samples from the renal transplant and control groups was probably due to the small number of samples size, conclusions about these results

Table 3: Distribution of BK polyomaviruses DNA positivity across PTP groups

RT-PCR/positivity	PTP		Total
	≤12	>12	
Positive			
Count	18	5	23
Percentage within RT-PCR/positivity	78.3	21.7	100.0
Percentage within PTP	24.0	16.1	21.7
Negative			
Count	57	26	83
Percentage within RT-PCR/positivity	68.7	31.3	100.0
Percentage within PTP	76.0	83.9	78.3
Total			
Count	75	31	106
Percentage within RT-PCR/positivity	70.8	29.2	100.0
Percentage within PTP	100.0	100.0	100.0
Chi-square test	0.371		

RT-PCR: Real-time polymerase chain reaction, PTP: Post-transplantation period

Table 4: Distribution of positive BK polyomaviruses DNA according to the immunosuppressive regimen in the renal transplant recipient group

RT-PCR/positivity	Immunosuppressive drugs		Total
	TAC	CYA	
Positive			
Count	19	4	23
Percentage within RT-PCR/positivity	82.6	17.4	100.0
Percentage within Immunosuppressive drugs	27.1	11.1	21.7
Negative			
Count	51	32	83
Percentage within RT-PCR/positivity	61.4	38.6	100.0
Percentage within Immunosuppressive drugs	72.9	88.9	78.3
Total			
Count	70	36	106
Percentage within RT-PCR/positivity	66.0	34.0	100.0
Percentage within Immunosuppressive drugs	100.0	100.0	100.0
P	0.05		

TAC: Tacrolimus, CYC: Cyclosporine, RT-PCR: Real-time polymerase chain reaction

Table 5: Distribution of positive BK polyomaviruses DNA in study groups according to serum creatinine

Serum creatinine	RT-PCR/positivity		Total
	Positive	Negative	
≤1.3			
Count	15	56	71
Percentage within serum creatinine	21.1	78.9	100.0
Percentage within RT-PCR/positivity	65.2	67.5	67.0
>1.3			
Count	8	27	35
Percentage within serum creatinine	22.9	77.1	100.0
Percentage within RT-PCR/positivity	34.8	32.5	33.0
Total			
Count	23	83	106
Percentage within serum creatinine	21.7	78.3	100.0
Percentage within RT-PCR/positivity	100.0	100.0	100.0
Chi-square test		0.839	

RT-PCR: Real-time polymerase chain reaction

are premature and may represent an epidemiological feature of the virus in Iraq, which may be related to the population density and environmental conditions, which are of paramount importance for BKV transmission.[16,17]

Studied groups in the present study were redistributed according to gender. In the patients' group, males were 67.0% according to sex there was no significant difference ($P \leq 0.005$). Such a percentage is coming in concomitance with several local studies. Al-Azzawi *et al*., 2019 study reported that 71.6% of patients were males.[9] Al-Obaidi *et al*., 2015 study mentioned a slightly higher percentage of males in the patient's group (78.79%).[13] Furthermore, a single-center 5-year study in Baghdad Medical City Teaching Hospital showed that the percentage of males in all cases of RTR was 62%.[17] Globally, several studies sited that males are more frequently subjected to kidney transplantations than females, due to variations in hormone levels. Men with elevated testosterone levels may have a decline in renal function. On the other side, estrogen may not protect men's kidneys which is more prevalent in women till menopause.[18] The age distribution in our study was 49.5% of them were equal to or <30 years while 50.5% were more than 30 years, and their mean age in the patient group was 36.88 ± 13.36 years, and in control groups 28.22 ± 7.95 ranging between 18 and 65 years. Such percentage and their mean were coming in associated with several local studies. Ali *et al*., 2016 study reported in the medical city teaching hospital, the mean age of the patient study was 34.07 ± 12.2 years, the youngest recipient was 9 years old, and the eldest was 66 years old.[17] Moreover, two previous studies reported in the Center of Kidney Diseases and Transplantation in the Medical City/Baghdad first showed the mean age of 30 healthy control group was 47.68 years, while in RTR patients was 48.14 ± 12.7.[10] Moreover the second study in this center, the mean age was 37 ± 13 years ranging between 18 and 67 years (Al-Obaidi *et al*., 2015).[13] Al-Azzawi, *et al*., 2019 study reported that the mean age of the individuals is 36.0 ± 13.8 years, the reason in the results mentioned above may be represent a small sample size.[9]

According to studies, BK viremia and BKVN are most prevalent in the 1st year following transplantation, when immunosuppressive is at its peak. This is in line with our results, which showed that (78.3%) 18 out of 23 had renal transplantation with equal to or <12 months ago. These findings are similar to those of (Al-Obaidi *et al*., 2015), who reported that (7%) 9 out of 12 patients who have kidney transplantation within below 12 months, and that some workers observed that BKVN can only emerge in rare circumstances within the 1st month following transplantation.[19] This is why we are looking for RTR patients with a posttransplant duration of 2–30 months, but not <2 months. BK viremia and the progress of BKVN are asymptomatic in the majority of patients, with the gradual increase in blood creatinine concentrations being the main sign that can be observed. To estimate the impact and the relative risk of BK viremia on renal function, which is represented by serum creatinine, the results show no differences neither in relative risk (OR: 0.904) nor in the distributions ($P = 0.839$). Moreover, this rise in serum creatinine frequently leads doctors to misdiagnose BKVN as an acute rejection or medication toxicity.[20]

The recipient variables were identified as the primary contributors to the indicated BK viremia risk factors (older age, male gender, inhibition of BKV-specific T-cell activity, and low dose of immunosuppressant drugs.[21] The findings of the current investigation revealed that out of 23/106 patients with RTR who tested positive for BK virus and were on an immunosuppressive regimen (either TAC or CYC-A [CSA]), the proportion of patients with BK virus was positive. Nineteen (82.6%) were on TAC and four (17.4%) were on CSA, so there was a statistically significant and relative risk of 1.344 which means are at a higher risk for TAC regimen than those on CSA regimen. This result agreement with that of AlObaidi *et al*., 2015, study in which 66.7% (8/12) of BK viremia of RTR patients were on a TAC regimen. TAC chemotherapy is a more potent immunosuppressive drug that inhibits the BKV-specific T-cell immune response, and a reduction in immunosuppressive drugs has resulted in an increase in the BKV-specific cellular immune response. TAC and CSA, on the other hand, were shown to decrease interferon expression by BKV-specific T cells in a dose-dependent manner.[13,22] From this study we can hypothesize that the only risk factor for BK viremia in the development of renal impairment is the overall degree of immunosuppression.

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Conflicts of interest

There are no conflicts of interest.

References

1. De Gascun CF, Carr MJ. Human polyomavirus reactivation: Disease pathogenesis and treatment approaches. Clin Dev Immunol 2013;2013:373579.
2. Peretti A, Geoghegan EM, Pastrana DV, Smola S, Feld P, Sauter M, *et al*. Characterization of BK polyomaviruses from kidney transplant recipients suggests a role for APOBEC3 in driving in-host virus evolution. Cell Host Microbe 2019;23:628-35.
3. Bennett SM, Broekema NM, Imperiale MJ. BK polyomavirus: Emerging pathogen. Microbes Infect 2012;14:672-83.
4. Boldorini R, Allegrini S, Miglio U, Paganotti A, Veggiani C, Mischitelli M, *et al*. Genomic mutations of viral protein 1 and BK virus nephropathy in kidney transplant recipients. J Med Virol 2009;81:1385-93.
5. Buck CB, Pastrana DV, Lowy DR, Schiller JT. Efficient intracellular assembly of papillomaviral vectors. J Virol 2004;78:751-7.
6. Loeches B, Valerio M, Pérez M, Bañares R, Ledesma J, Fogeda M, *et al*. BK virus in liver transplant recipients: A prospective study. Transplant Proc 2009;41:1033-7.
7. Buck CB, Thompson CD. Production of papillomavirus-based gene transfer vectors. Curr Protoc Cell Biol 2007. doi: 10.1002/0471143030.cb2601s37.
8. Carmichael GG. Gene regulation and quality control in murine polyomavirus infection. Viruses 2016;8:284.
9. Al-Azzawi TY, Al-Shawk RS, Al-Taee TJ. Immunohistochemical evaluation of renal allograft biopsies from a sample of Iraqi renal transplant recipients. Mustansiriya Med J 2019;18:97.
10. Shamran HA, Malik SN, Al-Saffer JM, Jawad RS. BK virus load associated with serum levels of SCD30 in renal transplant recipients. Int J Microbiol 2016;2016:9752097.
11. Al-Raisi F, Mohsin N, Kamble P. Management of BK virus nephropathy in kidney transplant recipients at the Royal Hospital – Clinical Audit – Oman. Exp Clin Transplant 2015;13 Suppl 1:156-8.
12. Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in104 renal transplant recipients. Am J Transplant 2005;5:1926-33.
13. Al-Obaidi AB, Abd KH, Kadhim HS, Habib MA, Abdlameer AS. BK polyomavirus and cytomegalovirus co-infections in renal transplant recipients: A single center study. Int J Adv Res 2015;3:856-64.
14. Mohammad TS. Detection of polyomavirus BK and JC in kidney transplant recipients. Iraqi Natl J Nurs Spec 2016;29:106-16.
15. Koukoulaki M, Grispou E, Pistolas D, Balaska K, Apostolou T, Anagnostopoulou M, *et al*. Prospective monitoring of BK virus replication in renal transplant recipients. Transpl Infect Dis 2009;11:1-10.
16. Trofe-Clark J, Sparkes T, Gentile C, Van Deerlin V, Sawinski D, Bloom RD. BK virus genotype variance and discordant BK viremia PCR assay results. Am J Transplant 2013;13:1112-3.
17. Ali AA, Al-Saedi AJ, Al-Mudhaffer AJ, Al-Taee KH. Five years renal transplantation data: Single-center experience from Iraq. Saudi J Kidney Dis Transplant 2016;27:341-7.
18. Soljancic A, Ruiz AL, Chandrashekar K, Maranon R, Liu R, Reckelhoff JF, *et al*. Protective role of testosterone in ischemia-reperfusion-induced acute kidney injury. Am J Physiol Regul Integr Comp Physiol 2013;304:R951-8.
19. Pakfetrat M, Malekmakan L, Torabinezhad S, Yousefi O, Naddaffard D. Review of renal biopsies, a single center experience. Iran J Kidney Dis 2020;14:12-9.
20. Mischitelli M, Bellizzi A, Anzivino E, Fioriti D, Boldorini R, Miglio U, *et al*. Complications post renal transplantation: Literature focuses on BK virus nephropathy and diagnostic tools available. Viro J 2008;5:38-43.
21. Barraclough KA, Isbel NM, Staatz CE, Johnson DW. BK virus in kidney transplant recipients: The influence of immunosuppression. J Transplant 2011;2011:750836.
22. Abend JR, Low JA, Imperiale MJ. Inhibitory effect of gamma interferon on BK virus gene expression and replication. J Virol 2007;81:272-9.