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BK Virus Infection: A Hidden Threat for Renal Transplant Recipients

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Abstract

BK virus (BKV) infection has become an important concern for renal transplant recipients, as it may cause nephropathy in transplant patients receiving immunosuppressive therapy resulting in renal dysfunction and possibly, graft loss. This cross sectional study was carried out at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2015 to June 2016, aimed to detect the incidence of BKV infection among transplant recipients from Bangladesh. A total of 30 randomly selected adult renal transplant recipients and 15 healthy controls were included in this study. Their blood and urine samples were collected at 4 and 12 weeks of post transplantation, and tested for BKV DNA by quantitative real-time polymerase chain reaction. The serum creatinine levels were measured along with other routine investigations at the Department Biochemistry, BSMMU. Virological analysis showed, 8 (26.6%) patients had detectable BKV DNA at 4 weeks (1 month). Of them, 23.3% (7/30) had viruria and 3.3% (1/30) had viraemia. No BKV DNA was detected either in blood or in urine samples of healthy controls. Incidence of BKV infection found significantly higher (p<0.02) in transplant patients than healthy controls. However, their serum creatinine value was not significantly higher than that of the BKV DNA negative patients. At third month (12 weeks post transplantation), BKViruria and/or viraemia were detected among 23.3% (7/30) patients where 13.3% (4/30) patients were newly detected who were previously (at 4 weeks of transplant) negative; only 1 (3.3%) patient had both viraemia and viruria. There was significant variation (p<0.05) in mean serum creatinine value of BKV DNA positive and BKV DNA negative recipients at third month follow-up. Significantly higher incidence of BKV infection among transplant patients indicates thatit is very likely occurring in transplantation recipients, andBKV screening test should be included in routine postoperative follow-up investigations for early detection; and thus prevent the graft loss due to BKV nephropathy.

Keywords:BK virus, Renal transplant, Serum creatinine, BKV DNA

Introduction

Human polyomavirus BK (BKV) was first identified from a Sudanese renal transplant recipients in 1971, since then it was thought to be a pathogen for renal transplant patients.[1] The clinical significance of BKV has been highlighted during the last two decades, with emerging evidence of its role in developing interstitial nephritis in renal allograft recipients.[2] BKV, as a ubiquitous virus, causes an asymptomatic infection during childhood that results in seroconversion in 80-90% of adult population and subsequently remains in a latent phase.[3,4] In immunocompromised hosts, specifically in renal transplant recipients, BKV reactivation could lead to invasive disease, where 5-10% may develop BKV nephropathy and approximately, 50% would suffer renal graft loss, if no intervention is attempted. In the late 1990s and early 2000s, BKV resulted an irreversible graft failure in 30 - 60% of cases.[2,5,6] Renal graft dysfunction is a late manifestation of BKV disease as no clinical symptoms are presented. Non invasive molecular methods can assist early diagnosis of BKV reactivation.[7]

Progression to BKV nephropathy occurs without clinical signs or symptoms, except for increasing serum creatinine concentrations over a period of weeks. As BKV persists in the kidney, the transplantation of organ from seropositive donors into seronegative recipients may also lead to BKV nephropathy. BK virus replication in the allograft has been correlated with the detection of BKV DNA in plasma by the polymerase chain

reaction (PCR) assay, thus BKV DNA may serve as a quantifiable surrogate marker of the course of infection.[8-10] With early detection of BK virus, the incidence of renal graft rejection can be reduced by decreasing immunosuppression (IS) to allow the immune system to control the infection, and this is the only method applied presently to prevent BK associated acute graft rejection. BKV persists in a nonreplicative latent stage in the renourinarytract.[11] In kidney transplant recipients, approximately 35- 47% of recipients develop BK viruria within 3 months of transplant and 20% develop BK viraemia within 12 months following transplantation.[12-14]

In Bangladesh, kidney transplantation is being done since the last three decades. Although BK associated nephropathy is one of the leading cause of transplant rejection worldwide, no information on BK virus prevalence in healthy people or among renal transplant recipients are available. There are neither systemic population based studies to estimate BK virus prevalence nor any guideline for detection of BK virus infection in transplant patients. Implementation of effectively organized screening programs is an urgent need to determine the burden of BK virus infection among transplant patients from Bangladesh. Early detection of BK virus can reduce the incidence of renal graft rejection. Thus, the present study was designed to detect BKV DNA among Bangladeshi renal transplant recipients.

Materials and Methods

This cross sectional study was carried out at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2015 to June 2016. A total of 30 randomly selected adult patients who had undergone successful renal transplantation in last one month at the Department of Nephrology, BSMMU and Kidney Urology Hospital of Dhaka city, and consented to participate in this study, were enrolled for sampling. Fifteen healthy controls were selected with their informed written consent and excluding impaired renal functions by biochemical tests and urine analysis. Participants from both groups found positive for hepatitis B, hepatitis C or human immunodeficiency virus were excluded from the study. Ethical clearance was taken from the 'Ethical Review Board' of BSMMU prior to the commencement of the study. Demographic data, immune suppression protocol, and serum creatinine levels were recorded in a semi-structured pre-tested questionnaire. With all aseptic precautions, 3 ml of venous blood and corresponding urine samples (10 ml of mid-stream urine) were collected from each patient at one month (4 weeks) of their transplantation. Same samples were taken from healthy controls. The patients were prospectively followed up at three months (12 weeks) of transplantation and both blood and urine samples were taken again from these patients. The collected blood and urine samples were centrifuged at 1400 rpm for 5 min and at 2300 rpm for 30 min respectively and supernatant of both samples were kept at -20°C with proper labelling for further analysis.

DNA extraction: DNA extraction of both plasma and urine was carried out using QIA amp DNA Mini Kit (QIAGEN, GERMANY) following modified method described by Pang et al.[15] DNA concentration was measured in ng/ µl by spectrophotometer (Nanodrop 2000/2000C) at the ratio of absorbance at 260 and 280 nm and the extracted DNA was stored at -20°C for real time PCR assay. Quantitative real-time PCR assay was performed by Step One™ PCR (Applied Bio system, USA) using real-time PCR kit (Geno-Sen's BK real time PCR kit,Genome Diagnostics, Netherlands) according to the manufacturer's protocol.[16] The cycling profile included: initial denaturation at 95°C for 10 minutes; denaturation at 95°C for 15 sec, annealing at 55°C for 20 sec and extension at 72°C for 15 sec (45 cycles). The results of the run were detected as signals/amplifications in fluorescence channel.

Data analysis for Real-time PCR: Data analysis was performed with ABI Step One™ Operator's manual. BKV DNA was determined based upon the Ct values for the sample BKV DNA and four standard curves resulting from analysis of

quantitation standard and the assay specific calibration coefficient. BKV DNA concentration was expressed in copies/ ml. The linear regression coefficient (R2) of the reference curve was maintained between 0.98 and 1.00.

Statistical analyses were carried out using SPSS 17.0 software. Chi-square test was used to compare the incidence of BKV infection among renal transplant recipients and control group.The confidence of interval (CI) of the calculated odds ratio (OR) was estimated by approximate 95%. For comparison of mean serum creatinine value, unpaired *t* test was done. *p* value <0.05 considered as statistically significant.

Results

Among 30 transplant recipients, males (90.0%) outnumbered females with mean age of 29 years (±7.49). Most of them belonged to middle income group. At first visit, a total of 21 (70%) patients had creatinine level within normal range (60-120 µmol/l) while 9 (30%) had creatinine level above normal range. Majority (80%) of healthy participants were females with their mean age 43.33 (±9.19) and serum creatinine within normal range (table I).

Table I: Demographic dataof Healthy control and Transplant recipients

Indicators	Number of Participants in Healthy control (n=15)	Number of Participants in Transplant recipients (n=30)
Sex		
Male	3	27
Female	12	3
Age		
Below 30	2	20
30-45	7	10
45+	6	0
Serum Creatinine level (1st followup)		21
Up to 120 µmol/L	15	9
Above 120 µmol/L	0	

Virological analysis of plasma and urine samples showed that 8 (26.6%) patients had detectable BKV DNA (Table II) at first month (4 weeks) of their transplantation. Of them, 23.3% (7/30) had viruria and 3.3% (1/30) had viraemia, which was significantly higher (*p<0.02*) among them as no BKV DNA was detected (table II).

Table II: BK virus DNA among healthy controls and transplant recipients

Study group		BK Virus DNA			
		Viruria n (%)	Positive Viremia n (%)	Total n (%)	Negative N(%)
Transplant Recipient (n=30)	At 4 weeks	07(23)	01(3.3)	08(26.3)	22(73.3)
	At 12 weeks	06(20)	01(3.3)	07(23.3)	23(76.7)
Healthy control (n=15)		0	0	0	15(100)

Chi-square test was done. *p* value <0.02.

The mean serum creatinine value of BKV DNA positive and DNA negative recipients was almost similar. These results were blinded to transplant physicians and no intervention of immune suppression was made before the third month sample collection.

At third month (12 weeks after transplantation), BKVviruria and/or viraemia were detected among 23.3% (7/30) patients where 13.3% (4/30) were negative in their previous samples. There was significant (*p*<0.05) variation of mean serum creatinine value of DNA positive and DNA negative individuals at third month (table-IV). Among newly detected patients, 3.3% (1/30) had both viruria and viraemia with an increase serum creatinine value (more than two fold of normal value), while another patient (3.3%) had high viral count in urine with three fold rise of serum creatinine value (table III).

Table III: Viral load and serum creatinine level of BKV DNA positive samples in prospective follow-up.

S L.	DNA count at 4 weeks (copies/ml)		DNA count at 12 weeks (copies/ml)		Serum creatinine (µmol/L) at 4 weeks	Serum creatinine (µmol/L) at 12 weeks
	Urine	Blood	Urine	Blood		
01	$3.18x10^2$	-	78.28	-	153.28	150.2
02	-	-	$5.38x10^5$	-	180.0	374.0
03	147.84	-	-	-	136.6	150.2
04		-	461.02		123.76	123.70
05	87.77	-	-	-	135.0	200.0
06	$2.70x10^2$	-	-	-	117.92	159.12
07	-	$1.17x10^3$	-	-	104.24	97.1
08	$3.77x10^2$	-	-	-	200.0	212.16
09	-	-	$1.32x10^3$	-	132.6	176.80

SL.	DNA count at 4 weeks (copies/ml) Urine	Blood	DNA count at 12 weeks (copies/ml) Urine	Blood	Serum creatinine (µmol/L) at 4 weeks	Serum creatinine (µmol/L) at 12 weeks
10	5.49x 10^2	-	9.94x10^7	-	143.90	148.0
11	-	-	1.90x10^2	7.92x10^3	137.4	272.0
12	9.31x 10^2	-	-	-	104.54	91.0

Table IV: Mean serum creatinine value of BKV DNA positive and BKV DNA negative transplant recipients in prospective follow-up.

	Mean S.creatinine level (mmol/l) BK Positive (Both Urine and Blood)	BK Negative (Both Urine and Blood)	P value
1st follow up	136.93	132.92	>0.10
2nd follow up	204.47	158.23	<0.05

Unpaired t test :*p* value <0.05 was considered as significant.

Discussion

Renal transplantation, a technologically advanced form of renal replacement therapy, now becomes the most acceptable mode of management for the patients with end stage renal disease (ESRD). Renal allograft recipients require permanent immunosuppression, and therefore, are at an increased risk for infections. Advances in immunosuppressive drug therapy has significantly reduced rejection related complications in renal allograft recipients, but the successful reduction of the immunity has been coupled with an increased incidence of BK viral infection among them, eventually leading to BKV nephropathy.[17] Early detection of BKV reactivation in the urine and plasma is a powerful clinical tool for identifying patients at risk for developing BKVN and for monitoring response to therapy.[18] Since the discovery of clinical significance of BK virus infection in renal transplant recipients, a great amount of scientific research has been taken place worldwide. However, there are no published data regarding BK virus nephropathy in renal transplant recipients or BK virus infection among healthy individuals and to the best of our knowledge, this is the first study of its kind from Bangladesh.

BK virus causes asymptomatic childhood infections which results in 80 to 90% of seroconversion and subsequently remains as latent state in adults in a variety of tissue sites, especially the kidneys.[19] The reactivation of latent virus in immunocompromised host may cause invasive diseases. Several studies report a variable incidence of BK viraemia and viruria ranging from 13 to 29% and 30 to 77% respectively in renal transplant recipients.[20,21] In this study, among 30 patients who had renal allograft transplantation in last one month, 23.3% (7/30) had viruria and 3.33% (1/30) had viraemia. As it is the first study of its kind in the country, there is no previous data to compare these results. However, these results are comparable to reports from neighboring countries. Studies from India detected viruria and viraemia respectively 17.6% and 5.44%, and 15.7% and 25% among post transplant patients.[17,22] A high prevalence 57% of BKV DNA excretion in the urine of renal transplant recipients has also been reported in a study from French.[13]Among seven patients with viruria in this study, two had DNA count >10^4 copies/ml of urine. In BKV reactivation, viral replication initially occurs in tubular cells of the kidney and causing cell injury, lyses, acute tubular necrosis, and subsequently gain access to the blood stream through injured tubular walls and via peritubular capillaries.[23] Thus, elevated levels of viral load in urine has a greater probability to develop viraemia, and the presence of BKV DNA in any sample either low or high count, indicate viral replication and thus need regular monitoring to determine the risk of development of renal damage.

BK viral load in plasma >10,000 copies/ml or urine viral load > 6.5x10^5 copies/ml that continues for at least four weeks, display 100% sensitivity of BK nephropathy diagnosis and such case should be considered as having presumptive BKV nephropathy.[12,24,25] In this study, at follow-up monitor after 12 weeks of transplantation (8 weeks interval after first visit), BKV was detected solely in urine of 20% (6/30) cases where 6.6% (2/30) had viruria at first month of visit. Among them, one patient had increasing DNA count from 5.49x10^4copies/ml to 9.94x10^7copies/ml during eight weeks of

interval. This persistence of viruria for more than four weeks and increasing viral load to>6.5x10^5 copies/ml, suggests that low-grade replication evolved into high viral replication, which need repeated follow-up and measurement of viral load to determine the risk of development of renal disease. Viruria is the first sign of active virus replication However, in some cases BKV DNA may be found in blood without findings in urine, suggesting that it remains latent in lymphocytes and the viraemia without viruria reflects the reactivation of BKV in circulating leukocytes.It was to be found that one case which had BKV viraemia but without any viruria.Similar findings were also reported where some cases did not follow the viruria-viraemia-nephritis sequence.[13, 26]

Serum creatinine level within normal range (50-120 mmol/L) is the most important marker of normal functioning kidney. In renal transplant patients, allograft dysfunction is presented with elevation of serum creatinine. In the present study, patients with viruria or viremia at first month did not show any sign of graft failure and their creatinine level did not change significantly.

Another study from Greece reported similar findings where patients with high plasma and/or urine viral load had stable renal function with no sign of graft failure.[27] However, progression to BKV nephropathy occurs without clinical signs or symptoms, except for increasing serum creatinine concentrations over a period of weeks while the serum creatinine level does not rise until at least half of the kidney's nephrons are destroyed or damaged.[28] For this, routine plasma and/or urine determination of BKV DNA load is more authentic than the creatinine value for early detection of BK viruria and progression to BKV nephropathy. However, in the follow-up visit at an interval of 8 weeks, our study found the mean creatinine value of BKV DNA positive recipients were significantly higher than that of the BKV DNA negative recipients. This finding may suggest the gradual deterioration of renal function due to persistent BKV infection. Detection of new 4 (13.33%) cases at 12 weeks follow up monitoring indicates that viral reactivation may potentially occur immediately at any point of time after a few months of transplantation. This observation may be supported by findings of various prospective studies which report that although the exact time of viral activation cannot be determined, it is more likely to commence in the early month of post transplantation.[12,24,27]

BKV typically remains latent in immune competent healthy adults and may be shed very rarely in the urine. Several studies reported 0.3% to 3.0% of immunocompetent adults may shed BKV in urine without clinical syndrome or other manifestations.[27, 29] However, in this study, BKV DNA was not detected either in urine or in blood of 15 healthy donors. Similar finding from immnunocompetent adult volunteers with no history of diseases associated with congenital or acquired immune deficiency or of treatment with antiviral or immunosuppressive drugs reported urine samples negative for BKV DNA by PCR.[29] Thus, findings from healthy controls of this study confirm published data that BKV remain inactive in the adult population with sound renal function.

Conclusion

BK virus infection has become an important concern for renal transplant patients worldwide. Early diagnosis and immunosuppression reduction accordingly may decrease the incidence of BKV infection induced graft failure. Although this study had certain limitations eg. the small number of participants and further follow-ups of the study population, the data obtained from this study indicates that BK virus infection is common among renal transplant recipients in the country. Further follow- up of BKV DNA positive patients may help to detect the role of BKV in developing nephropathy among Bangladeshi transplant recipients. In conclusion, it is of outmost importance to

determine BKV reactivation in the interim period of the first 6 months post-transplant to identify recipients at relative risk for acquiring BKV infection.

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References

1. Gardener SD, Field AM, Coleman DV, Hulme B. New human papovavirus (BK) isolated from urine after renal transplantation. Lancet.1971;19;1:1253-7.
2. Weikert BC, Blumberg EA. Viral infection after renal transplantation: surveillance and management. Clin J Am Soc Nephrol. 2008; 3(suppl 2): s76-s86.
3. Hirsch HH, Steiger J. Polyomavirus BK. Important review on BKV virology and pathogenesis. Lancet Infect Dis. 2003; 3: 611-23.
4. Doerries K. Latent and persistent human polyomavirus infection. Extensive review on persistent polyomavirus infection. In Khalili K, Stoner GL eds. Human Polyomaviruses. Molecular and clinical perspectives. New York: Wiley-Liss Inc. 2001: 197-237
5. Ferreira-Gonzalez A and Sidiqui R. BK virus in the transplant patient. Clin Microbiol News.2007; 29;16: 121-8.
6. Hirsch HH. Polyomavirus BK nephropathy: A(re-) emerging complication in renal transplantation. Am J Transplant. 2002; 2:25-30.
7. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, Mihatsch MJ, Nickeleit V, Ramos E, Randhawa P, Shapiro R, Steiger J, Suthanthiran M, Trofe J.Polyomavirus-associated nephropathy in renal transplantation:Interdisciplinary analyses and recommendations. Transplantation 2005; 79: 1277-86.
8. Mayar M, Nickeleit V, Hirsch HH, Dickenmann M, Mihatsch MJ, Steiger J. Polyomavirus BK nephropathy in a kidney transplant recipient; critical issues of diagnosis and management. Am J Kidney Dis. 2001; 383: e13.1-e13.5.
9. Nickeleit T, Klimkait T, Binet IF, Dalquen P, Del Zenero V, Thiel G, Mihatsch MJ, Hirsch HH. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med. 2000; 342:1309-15.
10. Limaye AP, Jerome KR, Kuhr CS, Ferrenberg J, Huang ML, Davis CL, Corey L, Marsh CL. Quantification of BKV load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. J Infect Dis. 2001; 183: 1669-72.
11. Hirsch HH, Mohampt M, Klimkait T. Prospective monitoring of BK virus load after discontinuing sirolimus treatment in a renal transplant patient with BKV allograft nephropathy. J Infect Dis. 2001;184:1494-5.
12. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. Am J Transplant. 2005; 5: 582-94.
13. Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. Am J Transplant. 2005; 5: 1926-33.
14. Sood P. and Hariharan S. BK virus infection after renal transplantation. Dial Transplant. 2011;40:102-7.
15. Pang XL, Doucette K, LeBlanc B, Cockfield SM, PreiksaitisJK. Monitoring of polyoma BK virus viruria and viraemia in renal allograft recipients by use of a quantitative Real time PCR assay: one year prospective study.J Clin Microbiol.2007; 45: 3568-73.
16. JC/BK Virus quantitative Real time PCR kit. Available at *www.professionalbiotech.net/pdf/jc-bk-virus.pdf.*
17. Badwal S, Chopra GS, Varma PP, Hooda AK. BK polyoma viral infection in renal allograft recipients.Med J Armed Forces India.2011;67:122-30.
18. Bechert CJ, SchnadigVJ, Payne DA, Dong J. Monitoring of BK Viral Load in Renal Allograft Recipients by Real-Time PCR Assays. Am J Clin Pathol .2010; 133: 242-50.
19. Heritage J, Chesters PM. McCance DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. J Med Virol. 1981; 8: 143-50.
20. Gardner SD, MacKenzie EF, Smith C, PorterAA. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J Clin Pathol. 1984; 37: 578-86.
21. Leung AY, Chan M,Tang SC, Liang R, KwongYL. Real-time quantitative analysis of polyoma BKviremia and viruria in renal allograft recipients. J Virol Methods. 2002; 103:51-6.

22. Thakur R, Arora S, Nada R, Minz M, Joshi K. Prospective monitoring of BK virus reactivation in renal transplant recipients in North India. Transpl Infect Dis. 2011; 13**:** 575–83.

23. Drachenberg CB, Papadimitriou JC, Wali R, Cubitt CL, Ramos E. BK polyoma virus allograft nephropathy: ultrastructural features from viral cell entry to lysis. Am J Transplant. 2003; 3: 1383-92.

24. Hirsch HH, KnowlesW, Dickenmann M et al. Prospective study of polyomavirus type BK replication and nephropathy in renaltransplant recipients. N Engl J Med. 2002; 347: 488-96.

25. NickeleitV, KlimkaitT, Binet IF, et al.Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med. 2000; 342: 1309-15.

26. Vats A, Shapiro R, Singh Randhawa P, Scantlebury V, Tuzuner A, Saxena M, Moritz ML, Beattie TJ, Gonwa T, Green MD, Ellis D. Quantitative viral load monitoring and cidofovir therapy for the management of BK virus–associated nephropathy in children and adults. Transplantation. 2003;75:105-12.

27. Koukoulaki M, Grispou E, Pistolas D, Balaska K, Apostolou T, Anagnostopoulou M, Tseleni-Kotsovili A, Hadjiconstantinou V, Paniara O, Saroglou G, Legakis N, Drakopoulos S. Prospective monitoring of BKvirus replication in renal transplant recipients. Transpl Infect Dis. 2009; 11: 1-10.

28. Rnceus.com.Serum creatinine.2016. Available at: http:// www. rnceus.com/renal/renalcreat.html.

29. Ling PD, Lednicky JA, Keitel WA, Poston DG, White ZS, Peng R, Liu Z, Mehta SK, Pierson DL, Rooney CM, Vilchez RA, Smith EO, Butel JS. The dynamics of herpesvirus and polyomavirus reactivation and shedding in healthy adults: a 14 month longitudinal study.J Infect Dis. 2003;187: 1571-80.