

BK virus excretion in acquired immunocompromised children: A comparison between kidney transplant recipients and steroid resistant nephrotic syndrome

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Background: BK virus (BKV) is ubiquitous in human beings. Virus reactivation may occur in immunocompromised settings. The aim of this study was to compare BKV excretion in acquired immunocompromised children (kidney transplant recipients and steroid resistant nephrotic syndrome) with normal population. **Materials and Methods:** One hundred and thirty one participants less than 20 years were recruited in the case-control study from June 2009 to December 2010. The participants consisted of 40 patients with steroid resistant nephrotic syndrome (subgroup 1), 39 kidney transplant recipients (subgroup 2) and 52 normal populations as control group. The first morning urine samples were analyzed in duplicate by conventional polymerase chain reaction (PCR) method for BKV. **Results:** Nine participants out of 131 had positive results for BKV. Three patients in subgroup 1 (7.5%), two patients in subgroup 2 (5.1%) and six people (11.5%) in the control group had positive PCR results for urinary BKV. No significant difference was noted among groups, $P = 0.53$. The mean of glomerular filtration rates in participants with positive and negative results for BKV were 125.5 ± 30.8 ml/min/m² and 132.2 ± 42.5 ml/min/m² respectively, $P = 0.8$. **Conclusion:** Acquired immunocompromised conditions did not increase the chance of urine BKV excretion in our study.

Key words: BK virus, child, kidney transplantation, Nephrotic syndrome, Polymerase chain reaction method

INTRODUCTION

BK virus (BKV) infection is one of the most common infections in kidney transplanted patients that causes emergent problems. BKV accompanies by JC (John Cunningham) virus and Simian virus 40 belongs to polyomaviridae family. The route of primary infection is through the upper respiratory tract. More than 80% of population changes to seropositive up to early adolescence.^[1,2] The incidence of intermittent reactivation and low levels of viruria of BKV oscillates between 5% and 10% in immunocompetent adults and 20-60% of immunocompromised individuals.^[3-5] There are few studies on urinary excretion of BKV in immunocompromised settings except for kidney transplantation. In this study we evaluated urinary shedding of BKV in children who have been receiving immunosuppressive medications (steroid, calcineurin inhibitors and mycophenolate mofetil [MMF]) for a long time comparing with normal population.

MATERIALS AND METHODS

This cross-sectional study was carried on 131 participants

at St. Alzahra hospital, Isfahan, Iran from June 2009 until December 2010. The case group consisted of 79 children and adolescents less than 20 years divided into two subgroups. Subgroup 1 included 40 patients with steroid resistant nephrotic syndrome (histopathology of focal segmental glomerulosclerosis, FSGS) and subgroup 2 contained 39 kidney transplant recipients. Fifty two normal populations were recruited in the study as the control group. The participants in subgroup 1 have been selected from all kidney transplant recipients less than 18 years who met the inclusion criteria. The patients in subgroup 2 have been recruited from those with confirmed diagnosis of FSGS who have been hospitalized at paediatric nephrology ward and also met the inclusion criteria. Control group was selected from normal adolescents who referred to the private clinics for routine examination.

Inclusion criteria for case group

- Glomerular filtration rate ≥ 90 ml/min/m²
- Elapsed time from kidney transplantation more than 6 months for subgroup 2
- Receiving steroid, MMF and cyclosporine/tacrolimus for at least 6 months in two subgroups

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- No episode of kidney rejection or receiving anti-thymocyte medications in recent 3 months in subgroup 2
- No proven urinary tract infection at the time of sampling.

Inclusion criteria for control group

- Glomerular filtration rate (GFR) ≥ 90 ml/min
- No past history of recent urinary tract infection (UTI)
- No past history of documented renal disease.

To increase the chance of detecting urinary epithelial cells, the first fasting urine samples were collected in duplicate. For each participant, at least 15 ml of urine specimen was taken out and centrifuged at 3500 rpm for 15 min. The sediments were frozen at -70°C before extracting DNA. Genomic DNA was extracted from the thawed samples by phenol/chloroform method. The extracted DNA in TE buffer was stored at -20°C until analysing by PCR method. The quality of the DNA was evaluated by PCR using beta-globin specific primers.^[6] PCR amplification was performed using specific primers of PEP-1 [5-AGTCTTTAGGGTCTTCTACC-3] and PEP-2 [5-GGTGCCAACCTATGGAACAG-3].^[7] Each PCR reaction was carried out in a total volume of 50 μlit containing, 1 \times PCR buffer, 1 mM MgCl_2 , 2.5 pMol of each primer, 0.2 mMol dNTP, 1.5 U Taq DNA polymerase (Sinagene, Iran), 10 μlit template DNA and distilled water to a total volume of 50 μlit . The PCR was performed in thermal cycler master (Eppendorf, Germany). The amplification condition was as follows: 94°C for 5 min; followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and a final extension at 72°C for 5 min. Purified BKV genome as positive and distilled water as negative controls were included in all runs. PCR products were analyzed on agarose gel electrophoresis followed by ethidium bromide staining. Definitive identification of BKV was accomplished by DNA sequencing. The results obtained from sequencing were compared with the BK genome sequences in Gene Bank by means of the Basic Local Alignment Search Tool (BLAST) program from The National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

The statistical software SPSS 17.0 was used for analysis. Data was analyzed by *t*-test and Chi-square.

RESULTS

Sixty-one out of 131 participants (46%) were female. The male to female ratio in case and control groups were 1.25/1 and 1/1 respectively [Table 1]. The mean of age for subgroup 1, 2 and control groups were 10.7 ± 5.6 years, 15.4 ± 4.3 years and 17.4 ± 7.5 years respectively. Approximately, 85% of patients in subgroup 2 have been received transplanted kidney from unrelated living donors and 15% from deceased donors. The median time of sampling after kidney transplantation was 36 ± 10 months. No kidney function deterioration was found in case groups until closing the study. Nine participants out of 131 had positive results for BKV. Three patients in subgroup 1 (7.5%), 2 patients in subgroup 2 (5.1%) and 6 people (11.5%) in the control group had positive PCR results for urinary BKV. No significant difference was noted among groups, $P > 0.05$. The mean of GFRs in participants with positive and negative results for BKV were 125.5 ± 30.8 ml/min/ 1.73 m^2 and 132.2 ± 42.5 ml/min/ 1.73 m^2 respectively, $P = 0.8$. Furthermore, the mean of GFR was not significantly different among all participants in 3 groups, Table 1.

DISCUSSION

In this study, we evaluated urinary excretion of BKV by PCR method in acquired immunocompromised children and adolescents (kidney transplant recipients and FSGS patients) and compared the results with immunocompetent population. According to our knowledge, it is the first study of its kind to compare BKV excretion in two groups of immunocompromised patients with kidney disease but normal GFR.

BKV and JCV are not uncommon in human beings. After almost always subclinical primary transmission in the infancy, these two viruses reside in uroepithelium with intermittent reactivation and low levels of viraemia (1, 3, 4 and 8). Different methods have been described to detect BKV in urine and serum and confirm BKV nephropathy. Among these methods, serial measurement of BK viral DNA in blood and urine by quantitative real time PCR, conventional PCR, end-stage quantitative PCR are useful in monitoring BKV nephropathy.^[8] We preferred quantitative PCR regarding its specificity, sensitivity and cost.^[8] It has been reported that 5-10% of immunocompetent

Table 1: Characteristics of the participants

| Participants | Gender (number) | | Age (year) \pm SD | GFR [ml/min/ 1.73 m^2] | Urinary BKV positivity (%) | Total | P value between groups |
|---------------|-----------------|------------|---------------------|----------------------------------|----------------------------|-----------|------------------------|
| | Male (%) | Female (%) | | | | | |
| Subgroup 1 | 23 (57.5) | 17 (42.5) | 10.7 ± 5.6 | 128 ± 26 | 3 (7.5) | 40 (100%) | $P > 0.05$ |
| Subgroup 2 | 21 (53.8) | 18 (46.2) | 15.4 ± 4.3 | 131 ± 29 | 2 (5.1) | 39 | |
| Control group | 26 (50) | 26 (50) | 17.4 ± 7.5 | 133 ± 31 | 6 (11.5) | 52 | |

Subgroup 1=Patients with steroid resistant nephrotic syndrome [subgroup 2] and 52; Subgroup 2=Kidney transplant recipients; GFR=Glomerular filtration rate; BKV=BK virus

adults may have intermittent viruria (1, 3 and 4). Latent BKV has been reported in more or less than 50% of native kidneys.^[9] In immunocompromised patients; the frequency of symptomless viruria reaches to as high as 60%.^[5,10,11] Hu and colleagues reported 4% and 5% symptomless viruria in renal transplant recipients with normal GFR and healthy blood donors, respectively.^[12] However, immunocompromised circumstances increase the risk of virus reactivation. A numerous of immunosuppressive protocols consisted of calcineurin inhibitors, MMF and steroids have been diagnosed as risk factors for reactivating latent viruses.^[13,14] Consuming these medications by kidney transplant recipients activates BKV followed by graft failure, mostly after the 1st year of transplantation.^[13,15-17]

High doses of steroids, cyclosporine (calcineurin inhibitor) and even MMF are frequently prescribed for most children with steroid resistant nephrotic syndrome. Therefore, theoretically, these patients are most susceptible to have reactivation of latent viruses such as BKV and JCV. Moran found that BKV reactivation is more common in pediatric renal transplant recipients comparing with children with primary nephrotic syndrome.^[18] In this study, both urine and serum samples were analyzed by real time PCR method. The author showed 37.5% positive urinary BKV in kidney transplants comparing with 24.3% in nephrotic syndrome. There was no significant difference in GFR between two groups. However, the patients with nephrotic syndrome were not limited to those with a history of receiving high doses of steroids, calcineurin inhibitors and MMF. In our study, the participants of case group were selected among patients who have been receiving similar immunosuppressive medications. Fogeda *et al.* reported BKV reactivation in 40 pediatric kidney recipients. They demonstrated different GFRs among patients with and without reactivation of BKV.^[19] We selected patients with normal GFRs and a medical history of similar treatment protocols containing steroid, cyclosporine/tacrolimus and MMF. The method that we used was conventional PCR. Although real time PCR is more sensitive, conventional PCR is a valuable tool in detecting BKV and distinguishing it from JCV. In addition, we increased the accuracy of gene sequencing by applying BLAST program from The National Center for Biotechnology to compare our results with the BK genome sequences in Gene Bank. However, we did not reveal an increase in BKV urinary excretion in kidney recipients and in FSGS children with normal renal function. Although the urinary BKV excretion was higher in control group, it was not significantly different with normal population. Nonetheless, the percentage of urinary BKV excretion in normal population was not greater than other studies (3, 4 and 11). Simultaneous urine and blood analysis of BK viral DNA by real time PCR may demonstrate more accurate results. We showed that urinary BKV excretion was

not significantly different between immunocompromised children and normal population. Although, urinary BKV excretion was higher in normal population, it was not noteworthy. The urinary excretion of BKV does not mean the BKV nephropathy or disease. In this study, we preferred to include kidney transplanted recipients and FSGS patients with normal GFR to avoid the possibility of BKV nephropathy and decrease the role of uremia in inducing BK viruria as an immune-compromising factor. Whether higher amounts of latent viral load in uroepithelium of some people comparing with others are responsible for attaining BKV nephropathy throughout immunocompromised situations, should be evaluated.

Limitations

The limitation of our study was the shortage of patients in each group. In addition, using real-time PCR is superior to conventional PCR that we used in our study. To diagnose BKV disease, detecting BKV in serum is necessary.

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REFERENCES

- Burgos D, Jironda C, Martín M, González-Molina M, Hernández D. BK-virus-associated Nephropathy. *Nefrologia* 2010;30:613-7.
- Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, *et al.* Polyomavirus-associated nephropathy in renal transplantation: Interdisciplinary analyses and recommendations. *Transplantation* 2005;79:1277-86.
- Polo C, Pérez JL, Mielnichuck A, Fedele CG, Niubò J, Tenorio A. Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. *Clin Microbiol Infect* 2004;10:640-4.
- Drachenberg CB, Hirsch HH, Ramos E, Papadimitriou JC. Polyomavirus disease in renal transplantation: Review of pathological findings and diagnostic methods. *Hum Pathol* 2005;36:1245-55.
- Drachenberg C, Hirsch HH, Papadimitriou JC, Mozafari P, Wali R, McKinney JD, *et al.* Cost efficiency in the prospective diagnosis and follow-up of polyomavirus allograft nephropathy. *Transplant Proc* 2004;36:3028-31.
- Borisch B, Caioni M, Hurwitz N, Dommann-Scherrer C, Odermatt B, Waelti E, *et al.* Epstein-Barr virus subtype distribution in angioimmunoblastic lymphadenopathy. *Int J Cancer* 1993;55:748-52.
- Arthur RR, Dagostin S, Shah KV. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. *J Clin Microbiol* 1989;27:1174-9.
- Costal C, Bergallo M, Ferrara P, Cavallo R. High-level polyomavirus JC viruria following long-term steroid therapy. *New Microbiol* 2010;33:405-7.
- Moriyama T, Sorokin A. BK virus (BKV): Infection, propagation, quantitation, purification, labeling, and analysis of cell entry. *Curr Protoc Cell Biol* 2009;Chapter26:Unit 26.2: 1-15.
- Jha V. Post-transplant infections: An ounce of prevention. *Indian J Nephrol* 2010;20:171-8.

11. Pang XL, Doucette K, LeBlanc B, Cockfield SM, Preiksaitis JK. Monitoring of polyomavirus BK virus viremia in renal allograft recipients by use of a quantitative real-time PCR assay: One-year prospective study. *J Clin Microbiol* 2007;45:3568-73.
12. Hu J, Zhao H, Huang Y, Zhang X, Gao H, Yang M, *et al.* Prospective study of posttransplant polyomavirus infection in renal transplant recipients. *Exp Clin Transplant* 2011;9:175-80.
13. Ramos E, Drachenberg CB, Portocarrero M, Wali R, Klassen DK, Fink JC, *et al.* BK virus nephropathy diagnosis and treatment: Experience at the University of Maryland Renal Transplant Program. *Clin Transpl* 2002; p. 143-53.
14. Hirsh HH, Friman S, Tuncer M, Wiecek A, Rostaing L. Prospective study of Polyomavirus, BK viremia and viremia in the novo renal transplantation. *Transplantation* 2006;82:4-1135.
15. Hirsch HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, *et al.* Polyomavirus-associated nephropathy in renal transplantation: Interdisciplinary analyses and recommendations. *Transplantation* 2005;79:1277-86.
16. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, *et al.* Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005;5:582-94.
17. Hirsch HH. BK virus: Opportunity makes a pathogen. *Clin Infect Dis* 2005;41:354-60.
18. Moran, Julie (Thesis), "Polyomaviruses in pediatric renal disease" (2008). Masters. Paper 44. Available from: <http://www.arrow.dit.ie/scienmas/44> [Last accessed 2013 Feb 24].
19. Fogeda M, Muñoz P, Luque A, Morales MD, Bouza E, BKV Study Group. Cross-sectional study of BK virus infection in pediatric kidney transplant recipients. *Pediatr Transplant* 2007;11:394-401.

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