

Original Article

BK and JC Polyomaviruses in Respiratory Specimens of Patients with Respiratory Disease

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Abstract

Background and Aims: The role of BK and JC polyomaviruses (BKV and JCV) in the causation of respiratory disease and the natural route of transmission has not well been established. The aim of study was to determine the prevalence of BK and JC viruses in 280 respiratory samples and evaluate their contribution to respiratory disease.

Materials and Methods: PCR was used to screen specimens for BKV and JCV and either single or multiplex RT-PCR, or real time PCR was used to determine co-infection with other viruses. Positive results were confirmed with sequencing.

Results: Of the 280 samples analysed, eight (2.85 %) were positive for BKV. BKV positive samples were from immunocompetent (n=5; 1.78%) and immunocompromised patients (n=3; 1.07%). The positive samples in the immunocompetent group were patients age range 8 days to 29 years. In the immunocompromised group, BKV positive patients age range 30 years to 69 years. Co-infections were found in 3 (37.5%) of the BKV positive samples. No sample was found to be positive for JCV.

Conclusion: Detection of BKV DNA in respiratory specimens supports previous studies suggesting the respiratory tract may be the primary site for acquisition or infection by BK virus at an early age and also reflect the reactivation of latent or persistent infection with the virus. Respiratory tract may not be considered as a site for JC viral persistent infection.

Keywords: Human polyomavirus; BK; JC; Respiratory infection; PCR; Co-infection

Introduction

Primary contact with polyomaviruses usually occur in childhood or early in adulthood. Little is known about the initial stages of polyomavirus pathogenesis in humans. The natural route of transmission has not yet been established. However, transmission studies carried out with animal polyomaviruses may give some indications.

For mouse polyomavirus, it seems that virus entry occurs by the respiratory route. A successful infection was established using a smaller inoculation of polyomavirus in the respiratory as compared to the alimentary tract [1], and primary viral replication was demonstrated in the respiratory tract and associated tissue [2]. BKV seroconversion has been linked to respiratory infections [3]. Seven sera from 177 examined sera taken from children with respiratory disease showed a seroconversion to BKV. Sundsfjord et al. [4] demonstrated BKV DNA, but not infectious BKV, in 2 of 201 nasopharyngeal aspirates from children with respiratory disease. From 259 nasopharyngeal aspirates analysed by Bialasiewicz et al. [5], 2 samples were positive

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for BKV DNA suggesting that alimentary tract should be considered as a portal of entrance to the human organism.

The site of JCV entry has not been well defined. JCV DNA was detected in 21 of 54 tonsil tissues, or 39% (15 of 38 children and 6 of 16 adults) by using regulatory-region primers and in 19 of 54 tonsil tissues, or 35% (13 of 38 children and 6 of 16 adults) by using the T-Ag primers [6]. In confirmation of Monaco *et al.* [6] findings, Kato *et al.* [7] detected JCV DNA in the tonsil tissue from 14 (44%) of 32 donors with tonsillitis. The authors suggested that primary infection occurs by respiratory inhalation and tonsils are the initial site of infection for JC virus.

In contrast, other studies failed to detect JCV DNA in respiratory specimens. Nasopharyngeal aspirates from 201 children with respiratory diseases and saliva from 60 human immunodeficiency virus type 1-infected adults and 10 healthy adult controls were analysed for the presence of JCV DNA by PCR [4].

They failed to detect any JCV DNA in either saliva or nasopharyngeal aspirates suggesting that JCV is not involved in causation of respiratory infections and neither salivary glands nor the respiratory tract are the site of JCV persistent infection. In confirmation of Sundsfjord *et al.* [4] findings, Bialasiewicz *et al.* [5] could not detect any JCV DNA in 259 examined respiratory specimens.

The aim of this study was to characterise the prevalence of BK and JC viruses in respiratory specimens collected from Tehran, Iran and evaluate either their site of entry or their contribution to respiratory disease alone or in combination with other viruses.

Methods

Specimen collection and processing: A total of 280 specimens from patients with respiratory tract disease were submitted to the Virology Laboratory between May 2016 and May 2017. The specimens were stored at -70°C until use. Specimens were re-used for this study. Specimens and associated clinical data were collected; the specimens were anonymised by

renumbering and removal of all patient identifiers from the data before use in this study .

Other virus screening: Specimens were extracted using the QIAamp® MinElute® Virus Spin kit (Qiagen) following the manufacturers instructions. To allow detection of RNA viruses, viral RNA was reverse transcribed to complementary DNA (cDNA) utilizing Applied Biosystems Taq Man® Reverse Transcription Kit (Applied Biosystems) and random hexamer priming prior to real-time PCR amplification using in-house assays for Influenza A, B, and C viruses; parainfluenza viruses types 1-3; PCRs for respiratory syncytial virus (RSV) types A and B, human bocavirus (hBoV) and human metapneumovirus (HMPV). For detection of the polyomaviruses KI, WU, MCV, SV40, and LPV in-house assays DNA PCRs were utilised as previously described [8, 9].

BKV and JCV PCR assays: Nucleic acid was extracted from nasopharyngeal aspirates using the QIAampDNA Blood BioRobot MDx Kit (Qiagen Ltd) according to the manufacturer's instructions and stored at -20°C until use. Nasopharyngeal samples (n=280) from patients with respiratory disease were tested by PCR using PEP1/PEP2 primers targeting large T antigen region of BKV and JCV genomes. To avoid cross-contamination, all pre-PCR processing was under taken in a separate location from PCR and post- PCR analysis. Standard precautions to avoid amplicon and nucleic acid contamination were taken. All samples were tested individually adding 5 µl nucleic acid to 45 µl master mix. Each reaction mix contained 200 pmol of each primer, 200 µM of dNTPs, 1 X PCR buffer, and 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR assays were performed on a GenAmp PCR System 9700 thermal cycler (Applied Biosystems). Samples were subjected to 1 cycle of 94° C for 10 min followed by 40 cycles of 95° C for 1 min, 56° C for 1 min, 72° C for 1 min and a final extension of 5 min at 72° C. Negative controls were included in each experiment. Amplified products were analysed by electrophoresis on 2% agarose gel, visualized with ultraviolet light (UV) light and

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compared for size with E-Gel Low Range Quantitative DNA Ladder (Invitrogen) .

Sequencing and phylogenetic analysis: Sequencing was carried out using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and the ABI 3100 Genetic Analyzer (Applied Biosystems). Sequences were assembled, analyzed, and edited using Sequencher software version 4.6 (Gene Codes Corporation, Ann Arbor, USA). Nucleotide sequences were aligned with Clustal W (University College Dublin, Eire). Phylogenetic analysis was performed on sequences derived from the VP1 region. All phylogenetic trees were visualized using Molecular Evolutionary Genetics Analysis (Mega) version 4.0 [10]. A bootstrap test with 1000 replicates was used to estimate the confidence of the branching pattern of the trees.

Results

Patient characteristics: Two hundred and eighty nasopharyngeal specimens from patients with respiratory disease were examined in the BK/JC polyomavirus assay. The median age of the 280 patients was 11 months (mean 9 years; range 8 days to 69 years), and the male to female ratio was 1.3:1. Specimens tested in this study were from 2 groups of patients; group I consisted of 223 nasopharyngeal specimens obtained from paediatrics patients who were immunocompetent (n=223; age range 8 days–29 years; mean 3 years; median 23 months). Group II included 57 nasopharyngeal specimens obtained from adult immuno-compromised patients (n=57; age range 30 years–69 years; mean 45 years; median 44 years). Both groups had upper or lower respiratory infection.

Prevalence of BKV and JCV: Nasopharyngeal samples (n=280) from patients with respiratory disease were tested by PCR using PEP1/PEP2 primers targeting large T antigen region of BKV and JCV genomes. Of the 280 samples analysed, 8 samples could be amplified by BK/JC PCR using the BKV and JCV specific primers (PEP1/PEP2). Positive samples were further investigated through bidirectional sequencing of their BK and JC assays ampli-

fication products. All positive samples in the BK/JC PCR assay showed identity with BKV sequences. Therefore, from 280 NPAs tested, 8 (2.85%) were found to be positive for BKV. No JCV positive sample was found.

Table 1. Age distribution of BKV infected patients with respiratory disease.

Age group (yrs)	Sample tested No.	BKV positive No. (%)
<1	140	3 (2.14)
1-5	43	1 (2.32)
6-14	20	1 (5)
15-29	20	0 (0)
30-44	23	0 (0)
45-60	21	1 (4.76)
>60	13	2 (15.38)
Total	280	8 (2.85)

Clinical findings associated with the presence of BKV infection: The age distribution of patients with BKV infection is shown in Table 1. BKV was identified in patients age range from 7 months to 69 years (mean: 14 years). The male to female ratio of the BKV positive patients was 0.28:1. More than two percent (2.14%) of children in the youngest age group (<1 year) were positive, and the figure rose to 5% at 6-14 years. This declined in the mid range years and thereafter rose to reach 15.38% at >60 years. BKV positive samples were from immunocompetent (n=5; 1.78%) and immunocompromised patients (n=3; 1.07%; Table 2). In the BKV positive immunocompromised group, one patient was a kidney transplant recipient and 2 patients were cancer cases. All BKV detections occurred during autumn (October; n=4) and winter (February; n=4). In three (37.5%) of the BKV positive cases, a co-infection with RSV-A (n=1), RSV-B (n=1), or a double co-infection with hBoV and Flu A (n=1) was detected. None of the BKV-positive samples were positive for influenza B and C, para-influenza viruses types 1-3, hMPV, SV40, LPV, MCV, KIV, and WU polyomavirus.

Table 2. Clinical presentation and demographics of BKV positive patients with respiratory disease.

Group	Sample tested No.	BKV positive No. (%)	Age range (mean)	Male/ Female	Respiratory disease	Co-detected viruses
Immunocompetent	227	5 (2.24)	8D-29Y (3 Y)	1/4	LRTI/URTI	RSV-B (n=1), RSV-A + Flu A (n=1),
Immunocompromised	53	3 (5.26)	30Y-69Y (45Y)	1/2	LRTI/URTI	RSV-B+hBoV (n=1)

Discussion

Two hundred and eighty nasopharyngeal aspirates from patients with respiratory diseases were analysed for the presence of BK and JC polyomavirus DNAs by PCR. From those, 8 (2.85%) were found to be positive for BKV as determined by BK/JC PCR assay (PEP1/PEP2) followed by sequencing. No sample was found to be positive for JCV.

The proportion of BKV positive patients obtained in this study is relatively higher than that found in previous studies, which detected BK virus in approximately 1% of patients [4-5]. This difference may be due to differential sensitivity of PCR, regional and temporal differences in the incidence of BKV infection, and different patient populations and samples types in the studies. Sequencing data of the BKV positive respiratory specimens revealed that the BKV found here was similar to the previously published sequences in GenBank including BK isolates from urine of renal, and bone marrow transplantation patients.

The data did not suggest any seasonal pattern. Co-infections with other respiratory viruses have not been reported in previous studies [4-5]. A relatively high proportion (37.5%) of co-infections with RSV-A, RSV-B, Flu A or hBoV was found. The true number of co-infections in our study is probably higher than the reported 37.5%, because several respiratory pathogens such as coronaviruses, rhinoviruses, enteroviruses and the adenovirus were not tested.

The study included 223 specimens from immunocompetent patients age range 8 days-29 years and 57 specimens from either transplant or cancer patients age range 30-69 years with

either lower or upper respiratory disease. BKV DNA was detected in 2.24% (n=5) of immunocompetent patients and 5.26% (n=3) of immunocompromised patients. The positive samples in the immunocompetent group were children age range 7 months to 29 years (mean=17 months). In the immunocompromised group (n=57; age range 30-69 years), BKV positive patients age range 30 years to 69 years (mean=47 years). In both groups, co-infection with other respiratory viruses was observed and the prevalence of viral infection was greater in females than males (M/F, 0.28: 1).

BKV reactivation has been observed in individuals with altered immune conditions including bone marrow transplantation, solid organ transplantations, leukaemia patients, autoimmune diseases such as systemic lupus erythematosus (SLE), and patients with the acquired immunodeficiency syndrome (AIDS) [11-12]. In support of previous findings, higher frequencies of BKV in immunocompromised patients (5.26%) may represent the reactivation of this virus in kidney transplant and cancer patients.

Detection of BKV DNA in respiratory specimens reported here supports previous studies (Bialasiewicz S. 2009) suggesting the respiratory system may be a transmission route for BK polyomavirus. In addition, the respiratory tract may be the primary site for acquisition or infection by BK virus at an early age in both immunocompetent and immunocompromised patients. However, the majority of BKV detections in respiratory samples coincided with the presence of other known respiratory pathogens, and therefore suggests

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that BK virus does not have a role in causation of respiratory disease.

The present study is in agreement with Sundsfjord et al. [4] and Bialasiewicz et al. [5] who failed to detect any JCV DNA in examined respiratory specimens. It is possible to suggest that JCV is not regularly associated with respiratory infections in patients with respiratory disease. The lack of JCV in children below 5 years of age in the study argues against the upper respiratory tract as a site for viral persistent infection or latency.

Conclusion

The respiratory system may be a transmission route for BKV. The respiratory tract may also be the primary site for acquisition or infection by BK virus at an early age.

Acknowledgment

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Conflict of interest

No conflict of interest

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References

1. Rowe WP. The epidemiology of mouse polyoma virus infection. *Bacteriol Rev.* 1961;25:18-31 .
2. Dubensky TW, Villarreal LP. The primary site of replication alters the eventual site of persistent infection by polyomavirus in mice. *J Virol.* 1984;50:541-546.
3. Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. *J Med Virol.* 1982;10:91-99 .
4. Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. *J Clin Microbiol.* 1994;32:1390-1394.
5. Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP. Detection of BK, JC, WU, or KI polyo-

maviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. *J Clin Virol.* 2009;45: 249–254.

6. Monaco MC, Jensen PN, Hou J, Durham LC, Major EO. Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. *J Virol.* 1998; 72: 9918-23 .

7. Kato A, Kitamura T, Takasaka T, Tominaga T, Ishikawa A, Zheng HY, et al. Detection of the archetypal regulatory region of JC virus from the tonsil tissue of patients with tonsillitis and tonsillar hypertrophy. *J Neurovirol.* 2004;10:244-249 .

8. Abedi Kiasari B, Valley PJ, Corless CE, Al-Hammadi M, Klapper PE. Age-related pattern of KI and WU polyomavirus infection. *J Clin Virol.* 2008;43:123-125.

9. Abedi Kiasari B, Valley PJ, Klapper PE. Merck cell polyomavirus DNA in immunocompetent and immunocompromised patients with respiratory disease. *J Med Virol.* 2011;83:2220-24.

10. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolution-ary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;24:1596-9.

11. Boudville N, Latham B, Cordingley F, Warr K. Renal failure in a patient with leukemia infiltration of the kidney and polyomavirus infection. *Nephrol Dial Transplantat.* 2001;16:1059-1061.

12. Shapiro S, Robin M, Esperou H, Devergie A, Rocha V, Garnier F, et al. Polyomavirus nephropathy in the native kidneys of an unrelated cord blood transplant recipient followed by a disseminated polyomavirus infection. *Transplantation.* 2006;27:292-293.