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Original Article

The Frequency of Human Polyomavirus BK in Patients with Systemic Lupus Erythematosus: A Cross-Sectional Case-Control Study

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Abstract

Background and Aim: Systemic lupus erythematosus (SLE) is an autoimmune disease and human polyomavirus BK (BKV) can be reactivated in patients with SLE due to the changes in the immune system and use of immunosuppressive drugs. In this study, we evaluated the prevalence of BKV infection among patients with SLE referred to Golestan hospital in Ahvaz, Iran between April 2013 to June 2016.

Methods: In this cross-sectional study we studied 75 individuals including 40 patients with SLE and 35 normal individuals. Urine and blood samples were taken and DNA was extracted from urine and plasma. Polymerase Chain Reaction (PCR) test was used to detect the BKV genome and positive samples were sequenced to confirm BKV. BioEdit software and MEGA 6.0 software were used for phylogenetic analysis to assemble the viral genome. A phylogenetic tree was constructed by neighbor-joining analysis with 1,000 replicates of the bootstrap resampling test using Mega 6.0. Statistical analysis was done by SPSS version 22.

Results: Among the 40 patients, 2 (5%) were men and 38 (95%) were women. The mean age of the patients was 39±10 years. 2.5% of plasma from patients with SLE were positive for BKV but none of the controls were positive in this regard.0% of control groups (p=0.346). Whereas in urine samples, 17.5% and 11.4% (p=0.458) of patients and the control group, were positive for BKV, respectively. However, there was no statistically significant difference between the patients and controls.

Conclusion: BKV reactivation occurs in 17.5% of patients with SLE during immunosuppression therapy. Therefore, more studies on BKV DNA by highly sensitive molecular assays in Patients with SLE seem to be necessary.

Keywords: Human polyomavirus; BK virus; Systemic lupus erythematosus; Renal, Polymerase Chain Reaction.

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Introduction

BK virus (BKV) and JC virus (JVC) are the two most commonly known human polyomaviruses, which were first described in the 1971s (1, 2). The BKV is a non-enveloped virus and it is approximately 45 nm in diameter. The virion contains a circular double-stranded DNA of approximately 5kbp (3, 4). The genome packed into particles is known to code for three structural proteins, such as viral capsid protein 1(VP1), VP2, and VP3 that VP1 is the major capsid protein (5). BKV is classified into four major subtypes including I (most frequent), II, III, and IV that variations in the DNA sequence of the VP1 gene are based on this division (6, 7).

Serological studies have shown that infection prevalence is widespread and occurs early in life. The BKV seropositivity reaching 90% in children aged 5 to 9 (8) and it's as high as around 90% in healthy adults (9). The BKV DNA has been found in 30 to 50% of normal renal tissues and 40% of ureters (10). Systemic lupus erythematous (SLE) is an immune-mediated disease disorder in which the

patients experience several complications such as renal problems (11). The hormonal and immunological changes, inflammation, and infections can exacerbate the immune system responses and increase the risk of SLE especially in pregnancy (12). The immune system change in autoimmune diseases such as SLE and the use of immunosuppressive drugs have been confirmed (13). In SLE disease, BKV which is linked with several implications especially in renal such as ureteral stenosis, hemorrhagic cystitis, and nephropathy, can be reactivated and implicated in the disease severity. Furthermore, the virus is associated with pneumonitis, retinitis, liver disease, and meningoencephalitis (14, 15). Asymptomatic BK viruria occurs in up to 60% of immunocompromised patients (16). Use of immune-modulatory drugs seems to increase the prevalence of BKV to 63% in Patients with SLE. However, the prevalence rate before starting Natalizumab and Rituximab seems to be 8.3% and 27% respectively (17). BKV prevalence reported in 1 to 15.7% of plasma and 41.8% of urine samples of kidney transplanted patients in Iran (18). In this study we evaluated the existence of BKV in patients with SLE in Ahvaz a city at south west of Iran between April 2013 and June 2016 and compared with a control group to make a comparison and find out if SLE patents are more vulnerable to BKV.

Methods

Study design

In this study 75 individuals including 40 patients with SLE that were selected by rheumatologists based on clinical and laboratory characteristics from April 2013 to June 2016 in Golestan Hospital, Ahvaz city were studied and 35 healthy individuals were selected as a control group. About 50 mL urine was collected in a falcon that contained 0.5 mL of 0.5 M EDTA, pH 8.0. Each sample was centrifuged at 3000 rpm for 5 minutes and the pellet was kept at -80°C until further analyses. Also, 3–5 mL of blood was collected in single EDTA tubes. Specimens were centrifuged for 5 min at 3000 rpm. Plasma samples were collected and then stored at -80◦C until further analyses.

Extraction of DNA from plasma and urine

DNA was extracted from 200 µL of urine and plasma specimens using High Pure Viral Nucleic Acid Kit (Roche, Germany) according to manufacturer's instructions. The extracted DNA was stored at -20°C till used.

PCR

The following primers were used for the detection of BKV DNA:

Forward primer, 327-1PST (5′-GCCTGCAGCAAGTGCCAAAACTACTAAT-3′; nt 1630–1649); Reverse primer, 327-2HIN (5′-GCAAGCTTGCATGAAGGTTAAGCATGC-3′; nt 1956–1937) (19).PCR was performed with 25 µL volume, containing the 3 µL extracted DNA, 0.5 µL from each primer, 12.5 µL amplification premix (PCRBIO Taq Mix Red 2x) and 8.5µl distilled water. The reaction mixture was subjected to a thermal cycler (PeQlab, Germany) with the following program: 1 cycle with an initial denaturation at 95°C for 2 minutes, followed by 40 cycles, 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and final extension at 72°C for 5 minutes. All reactions were performed in the presence of negative and positive controls. The 342 bp PCR product was subjected to electrophoresis on a 1.7% agarose gel supplemented with DNA safe Stain (Cinna Gen) and visualized using a UV transilluminator.To determine the BKV subtype, positive samples were sequenced by Noor gene laboratory, Ahvaz, Iran.

Phylogenetic study

All sequenced viral DNA fragments were assembled using the BioEdit software. The MEGA 6.0 software was used for phylogenetic analysis. The partial nucleotide sequences of VP1 (342 nt) aligned with the different BKV genotypes isolated from different regions of the world by CLUSTAL W. Phylogenetic trees were constructed by neighbor-joining analysis with 1,000 replicates of the bootstrap resampling test using Mega 6.0.

Ethical consent

The project was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz Iran. All of the individuals included in the study were orally informed about

the purpose of the study and invited to participate. Each patient gave informed consent.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA). The Chi-Square test was used to calculate. $p<0.05$ was accepted as significant.

Results

From the total of 40 patients, 2 (5%) were males and 38 (95%) were females. In the control group, all individuals (100%) were female. The mean age of the patients was 39±10 years. The mean age of the control group was 41±8 years. All individuals were between 19 and 63 years old.

Overall, among our 75 cases, we found BKV viremia in one case (2.5%) and viruria in 11 (12.9%). One out of 40 patients was positive for BK virus in plasma samples (2.5%). In the control group, no positive cases were detected. The prevalence of the BK virus was not significant between both groups (p= 0.346).

Seven out of 40 (17.5%) patient's urine samples were positive for BKV. In the control group, 4 out of 35 (11.4%) cases were positive and there was no significant difference (p= 0.458). The above items are shown in Table 1. Four out of 12 positive samples were "subtype I" BKV base on Sanger sequencing technique (Figure 1).

Table 1: BK viremia and viruria in 40 patients with systemic lupus erythematosus and 35 healthy individuals

Group	BK DNA in plasma		p-value	BK DNA in urine		p-value
Patients N= 40	Positive	N=1 (2.5%)	0.346	Positive	N= 7 (17.5%)	0.458
	Negative	N= 39 (97.5)		Negative	N= 33 (82.5%)	
Control N= 35	Positive	N= 0 (0%)		Positive	N= 4 (11.4%)	
	Negative	N= 35 (100%)		Negative	N= 31 (88.6%)	

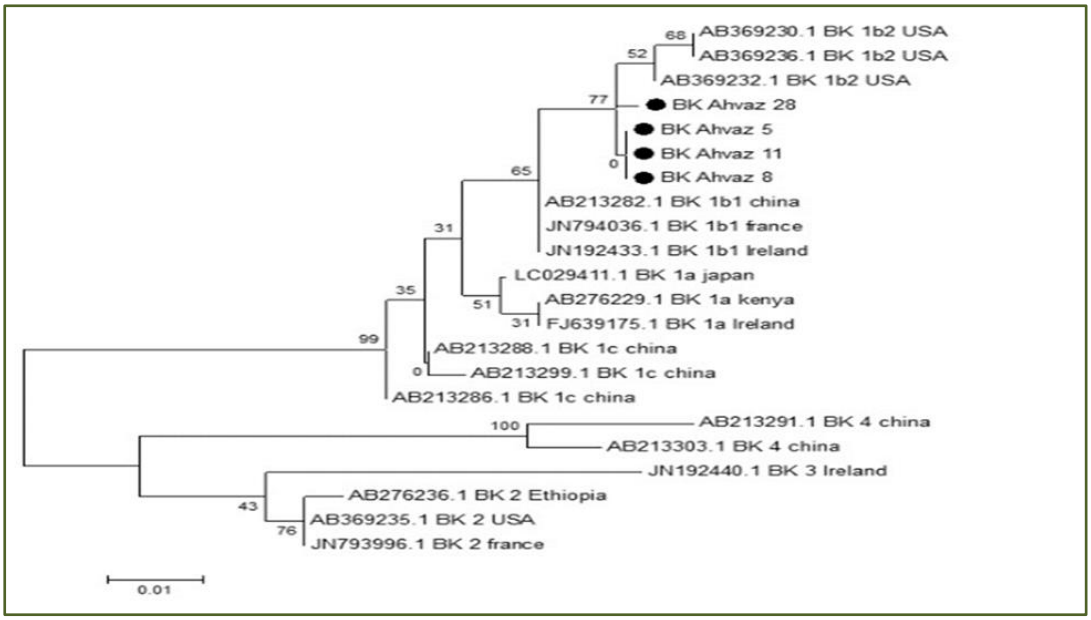

Figure 1. A phylogenic tree was constructed with neighbor-joining method using the partial sequences of VP1 region of BKV, reference sequences were retrieved from GenBank with their Accession Numbers. The results of the phylogenetic tree show the isolated Iranian with circular black are clusters with BKV 1b accession number AB369232.1 isolated from the USA. The accuracy was assessed by 1000 bootstrap replicates. Scale bar=0.01

Discussion

The human polyomaviruses BKV are the most prevalent viruses in the human population worldwide (20). The polyomavirus infection rates might be variations between different countries (21). There is a significant association between the BKV infection and SLE disease development (3).

Impairment of the human immune system due to the use of immunosuppressive drugs or deregulated immune responses are the potential risk factors for BKV reactivation in Patients with SLE (22). Immunosuppressive drugs, especially cyclosporine A, have been reported to increase Transforming Growth Factor (TGF)- β1 expression, which in turn leads to immune suppression. Studies indicated that TGF- β1can enhance BKV reactivation in renal tubular cells in cell culture. In patients with SLE' immune complexes in serum (anti-double-stranded DNA-dsDNA) often correlated with inflammation of the kidneys and leads to lupus nephritis (23). T antigen is the BK virus' transcription factor and is a DNA-binding protein that binds to both viral and host cell DNA (24). Therefore, the role of BKV in Patients with SLE can be examined in two aspects. 1) BKV can lead to induced lupus syndrome or 2) the use of immunosuppressive agents and biological therapies that can increase the reactivation of BKV infection and affect the prognosis and treatment of SLE. BKV is detectable in the blood (whole blood, plasma, serum), urine, renal tissue via detection of viral cytopathic effect (decoy cells), NAT, and BKV specific antibody (25). Usually, the first indication of the reactivation of BKV is BK viruria which subsequently occurs after several weeks of the BK viremia (7). The prevalence of BK in renal transplant recipients is approximately 1% to 10% and viruria and viremia precede nephritis might happen as early as 6 days and up to 5 years posttransplant. Viruria and viremia can be detected weeks to months before a detectable increase in serum creatinine levels among transplant candidate (26).

In Japan, Taguchi et al. showed the presence of BKV antigen via indirect immunofluorescence technique in the urine of two patients with SLE (27). In Norway, Sundsfjord et al. analyzed 44 DNA extracted from urine samples using the PCR method in patients with SLE and healthy individuals. Their result indicated that 7 (16%) of 44 patients and 0 (0%) of 88 matched healthy controls were positive for BKV DNA. About 26 % of those patients continued to have persistent or recurrent BK viruria at 1–3 years follow-up (28). This study is parallel with our study and the results are strikingly resemblance to ours.

In another study in Norway, Bendiksen et al. used PCR methods to examine urine samples of 19 patients with SLE and they reported 17(89.4%) positive cases for BKV. During a year follow-up study, BKV was detected in 48 (48%) of 100 specimens (29). In Italy, Colla et al. confirmed the presence of BKV DNA by semi-nested PCR in serum and urine specimens of 40 SLE nephritis patients and 29 healthy controls. In this study, BKV DNA was detected in 15% and 13.8% of serum samples of patients and the control group, respectively, as well as, 32 % and 17.2% of urine samples (30). In Taiwan, LU et al. was tested the urine samples of 95 patients with SLE and 32 healthy individuals for BKV DNA by real-time PCR. The results of this study showed a high prevalence rate of BKV viruria in patients (71.6%) than in the control group (18.6%). Their results suggest the possibility of immunosuppression in BKV reactivation in the urinary system. They have also observed a significant correlation between the reactivation of BKV and platelet count (31). In Thailand, Rianthavorn et al. indicated 32% (16 of 50) of urine samples of pediatric patients with SLE were positive for BKV DNA via nested PCR (32). In the USA, Gupta et al. reported the prevalence of BKV in children with SLE was 3.1% (1 of 32) for BK viruria and 6.2% (2 of 32) for BK viremia (17). This controversy may come from the differences in the genetic background of the patients, differences in the age of patients, differences in the sample size, and differences in the diagnostic methods. However, this study has several limitations. Firstly, since different phases of SLE disease and its treatment can cause a major susceptibility to develop BKV infection and virus reactivation occurs during impaired immune conditions, it was better to take several samples from the patients

during the treatment course. Secondly, the number of cases tested in this study was low; hence, further studies with larger populations are needed to understand the impact of immunosuppression in patients with SLE and the risk for BKV infections. Thirdly, the use of more sensitive methods for detecting such as nested PCR or Real-time PCR is recommended. Lastly, the measurement of urea as an important potential inhibitor of the PCR and assessment of routine viral load monitoring is suggested for future studies.

Conclusion

In the present study, the prevalence of BKV was determined in serum and urine samples using PCR. Here, the BKV viremia was observed among 2.5% versus 0% and BK viruria in 17.5% versus 11.4% of the patients and control groups, respectively. Our results show that the all our subtype is subtype I of BKV which is in accordance with other reports. Finally, this study showed a low rate of BK viremia and viruria in patients with SLE, and prevalence data in those patients did not significantly differ from the control group. Since BKV is closely linked to renal failure, more comprehensive studies are needed to prove it in Patients with SLE.

Conflict of Interest

The authors declared that they have no conflict of interest.

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Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee in Ahvaz Jundishapur University (IR.AJUMS.REC.1397.051) in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author Contribution

S. B. S. performed the experiment, analyzed the data, and wrote the paper. Gh. K suggested the research idea, technical and material support, edited the manuscript, and study supervision. M. M. contributed to developing the research design. E.R assisted in the diagnosis of patients. K.A. performed statistical analysis.

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