

Different Strains of BK Polyomavirus: VP1 Sequences in a Group of Iranian Prostate Cancer Patients

Maryam Vaezjalali^{1,2}, Helia Azimi^{1,3}, Seyed Masoud Hosseini³, Afsoon Taghavi⁴, Hossein Goudarzi^{2*}

Purpose: BK virus (BKV) has a worldwide seroprevalence in humans. Based on sequences of the major capsid proteins, i.e. viral protein 1 (VP1), there are four BKV genotypes. Each genotype has its own subtypes, and was shown to be circulating independently in the human population. The aim of this study was to determine BKV genotypes and subtypes among Iranian patients with prostatic cancer, benign prostatic hyperplasia, and kidney transplantation.

Materials and Methods: BKV DNA was extracted from prostatic cancers and benign prostatic hyperplasia blocks and also urine of kidney transplantation patients. BKV (VP1) gene was amplified partially (327nt) by homemade polymerase chain reactions and subjected for sequencing and phylogenetic analysis. Bioedit version 7.0 and Mega version 5.0 were used for sequence analysis and for comparing the results with world-driven BKV sequences.

Results: All of BKV VP1 genes which were derived from Iranian patients were classified with subtype 1b2 strains from Germany and Turkey. Predicted amino acid sequences from the studied region of VP1 showed that all of these nucleotide diversities could change amino acid sequence numbers 60, 68, 72, 73 and 82 among VP1.

Conclusion: The interesting point was that genetic analysis of derived sequences showed a different feature of genetic diversity among Iranian sequences. This feature has not been reported yet. This characteristic feature of Iranian BKV VP1 gene provides a unique cluster of sequences in phylogenetic tree.

Keywords: BK virus; agnoprotein; genotype; prostate cancer; benign prostatic hyperplasia.

INTRODUCTION

BK virus (BKV), a member of Polyomaviridae, is ever-present among humans⁽¹⁾. In western countries, BKV infects children asymptotically^(2,3). About 50% of children have BKV antibodies by four years old. By the age of 10 years old, 90-100% of them have seroconverted. BKV may persist in kidney tissue^(4,5) and its contribution to human cancers is controversial. This virus has a double-stranded circular DNA genome of about 5 kbp packaged within a capsid of 45-50 nm in diameter⁽⁶⁾. The BKV genome is divided into regulatory, early, and late regions, and encodes five major proteins^(7,8). The capsid contains three proteins: viral proteins (VP) 1, 2 and 3.

VP1 gene is responsible for coding VP1, a major structural protein that comprises approximately 80% of the total viral capsid protein. The VP1 protein has important domains which interact with viral receptors on host cells. A single amino acid change in VP1 protein can increase pathogenicity of mouse polyomavirus⁽⁹⁾. Diversities of VP1 gene lead to significant variation among BK viruses worldwide⁽¹⁰⁾.

BKV isolates are classified into four subtypes (I-IV) worldwide using serological and genotyping methods^(1,11). Subtype I is prevalent throughout the world, sub-

type IV is in Asia and part of Europe, and subtypes II and III are rare in the world. These four genotypes correlate well with four serogroups, and were shown to be circulating independently in the human population.

There are three reasons for researching about BKV genotypes and subtypes: 1) There is little information about the geographical circulation of BKV subtypes and subgroups. The effect of each genotype or subtype on clinical implications has previously been suggested^(12,13); 2) Some researchers had reported that characterization of BK virus' genetic mutations has biological and clinical implications⁽¹⁴⁾. This data may be needed to follow potential relationships between BKV genotype and clinical disease in future; and 3) Diagnostic virology laboratories need the sequencing data of BKVs to ensure correct detection of all naturally occurring viral strains⁽¹⁵⁾.

This study tried to explain phylogenetic characterization of BKV strains among Iranian population and analyze its different polymorphisms clinically. The BKV VP1 region was studied among Iranian patients with prostatic cancer, benign prostatic hyperplasia, and kidney transplantation.

MATERIALS AND METHODS

Twenty-five samples from our previous work on BKV

¹ Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

² Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

³ Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.

⁴ Department of Pathology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Correspondence: Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Velenjak St., Tehran, Iran. Postal Code: 1985717443. Tel: +98 21 23872556. Fax: +98 21 22439972. Mobile: +98 9126194134.

E mail: Hossein.Goudarzi@sbmu.ac.ir.

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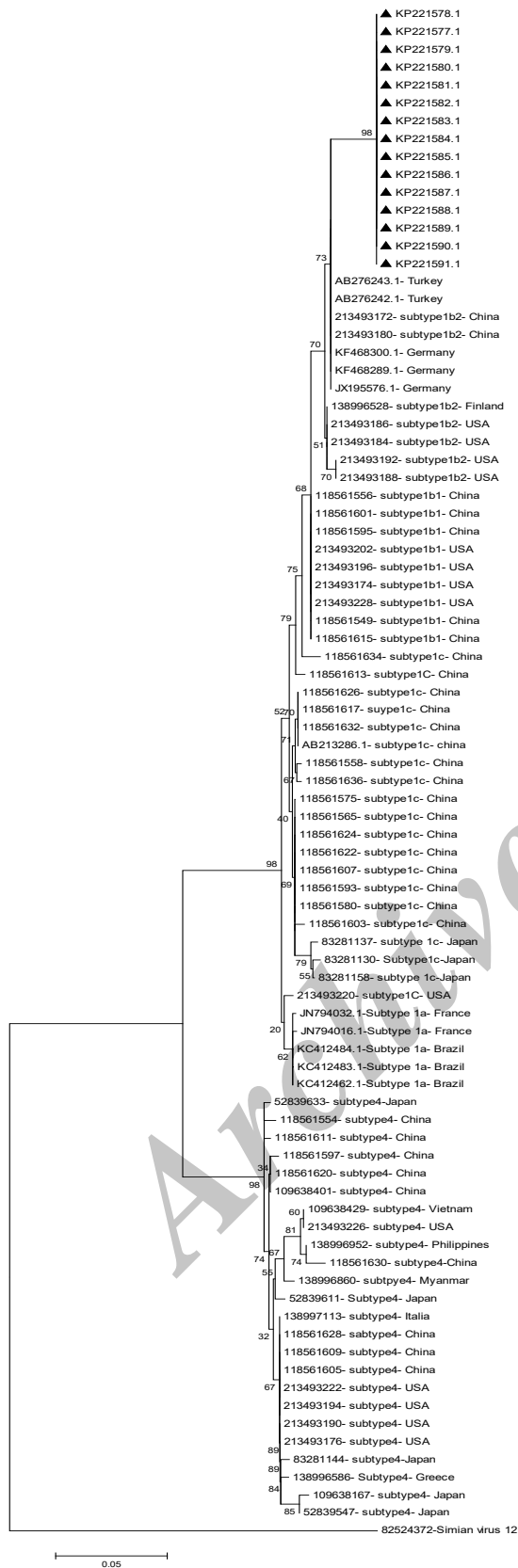


Figure 1. Phylogenetic tree of BKV VP1- gene partial sequence constructed using 15 isolates from this study along with Genbank reference sequences. SA12 (a primate polyomavirus related to BKV) was considered as the outgroup.

were studied in this investigation⁽¹⁶⁾. All patients were referred to a central hospital in Tehran. Each test was done considering one specimen as one patient.

This study was approved by the Ethics Committee of the Urology and Nephrology Research Center (UNRC approval number 12-22 on 2012/3/12) and is in accordance with the Helsinki declaration of 1964.

DNA extraction, gene amplification and sequencing DNA was extracted from urine samples with QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and also from paraffin-embedded tissue blocks with QIAamp-DNA FFPE Tissue extraction kit (Qiagen, Duesseldorf, Germany). The protocols for working on the samples were followed based on our previous study (16). Briefly amplification of beta-globin was done for all samples to control DNA extraction. Then BKV amplification was done with specific primers⁽¹⁶⁾. All experiments were done in a contamination-free environment.

For each round of polymerase chain reaction (PCR), positive and negative controls were considered. PCR program included five minutes of denaturation at 94°C, followed by 35 rounds of an amplification cycle consisting of 35 seconds denaturation at 94°C, one minute annealing at 50°C for beta-globin and 55°C for BK virus and one minute extension at 72°C, and a final extension cycle of four minutes at 72°C. Beta-globin and BKV positive samples were detected with gel electrophoresis and etidium bromide dyeing of 268 and 327 bp PCR product, respectively. One separate BK virus positive specimen from the urine of infected patients was used as positive control. One beta-globin and all BKV PCR products were purified by QIAquick PCR purification kit (Qiagen, Duesseldorf, Germany). Then, they were subjected for direct sequencing (Genetic Analyzer ABI- 3130 DNA Sequencer, Fostercity, CA, USA) with 10 pmol of related PCR primers bi-directionally.

Phylogenetic analysis

Sequences derived from samples were read by Chromas software and aligned with reference sequences by Bioedit version 7. Their phylogenetic tree was drawn by Mega 5.0 software. The evolutionary history was inferred using the neighbor joining method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the analyzed taxa. The evolutionary distances were computed using the Kimura two-parameter method and were in the units of the number of base substitutions per site. A neighbor joining phylogenetic tree was reconstructed from the typing-region sequences obtained from Iranian patients plus 88 reference sequences, using SA12 (a primate polyomavirus related to BKV) as the outgroup.

RESULTS

The patients from whom the samples were driven were from 10 provinces of Iran. All of them were men with a mean age of 68.0 ± 8.9 years old (range: 50 to 95 years old). Nephropathy of BKV was not reported.

Also, derived sequences from one beta-globin PCR product confirmed the presence of beta-globin gene. Among 25 positive BKV samples, 15 samples were successfully sequenced. Nine samples were derived out from prostatic cancer tissues and five were derived out from benign prostatic hyperplasia tissues. Also, one sample was derived from a patient with kidney transplantation who was receiving immunosuppressive med-

ications.

In the resulted tree (**Figure 1**), typing-region sequences were divided into clusters corresponding to subtype I and all of Iranian BKV strains were clustered with subtype 1b2 strains from Germany, Turkey and USA. It was apparent that Iranian BKV sequences are classified as a different cluster among other subtype 1b2 strains. The sequences derived from this study have been submitted to GenBank by accession numbers: KP221577-KP221591.

In this study nucleotides 118-393 and therefore predicted amino acid 40-132 of VP1 were investigated. Iranian strains alignment with reference sequence (accession number: JX195576.1) showed that five nucleotides were different among the derived VP1 region, 180, 202, 215, 218 and 244. The predicted amino acid sequences from the studied region of VP1 showed that all of these nucleotide diversities could change amino acid sequence numbers 60, 68, 72, 73 and 82 among VP1.

DISCUSSION

In this study, phylogenetic analysis was done on 327 nucleotides of BKV VP1 region derived out from 15 patients. There were two remarkable findings. One was that phylogenetic analysis of these sequences established one cluster for designated Iranian sequences. This cluster was classified with high bootstrap value with BKV sequences subtype 1b2 from Turkey and Germany. Subtype 1b2 is of type 1 which had been reported in Europe previously⁽¹⁵⁾. Subtypes a, b1 and c are other subgroups of subtype 1 which are most prevalent in Africa, South-east Asia and North-east Asia, respectively⁽¹⁷⁻²⁰⁾. Two previous studies in Iran had detected BKV subtypes among kidney transplant recipients by restriction fragment length polymorphism - polymerase chain reaction (RFLP-PCR).

In 2012, researchers reported BKV subtype 1 among 12 Iranian-Azeri people from north-west of Iran⁽¹²⁾. In 2015, researchers reported BKV subtype 1 from east of Iran⁽²¹⁾. From 51 BKV samples in that study, 94.11% were subtype I and 5.89% were subtype IV using the RFLP method. None of the patients' urine samples were positive for subtypes II and III. One reason for achieving different results in our study might be because of fewer samples, ethnicity or different clinical samples. However, sequencing is the standard test for genotyping which can help researchers analyze viral sequences sufficiently. On the other hand, in a new study, BKV subtype 1b2 was reported among Iranian HIV-infected patients⁽²²⁾.

The other significant point was that our studied sequences were obtained from different clinical sources and geographical regions of Iran. Despite controlling PCR conditions and preventing DNA contamination, no difference was observed between strains derived from patients with prostate cancer, benign prostatic hyperplasia and kidney transplantation. All of the derived sequences belonged to BKV subtype 1 in this study. Previous studies had reported that despite different clinical samples, subtype 1 was predominant around the world⁽²³⁻²⁵⁾.

Since BKV subtype I was geographically prevalent, some researchers had suggested that there is no significant correlation between BKV subtypes and geographical regions⁽¹⁷⁾. However, it seems that different research design of studies might be the cause of this theory. Previous studies had reported 287 bp of VP1 as a remark-

able region for BKV genotyping. Two articles reported that full genome sequencing can detect viral genotypes more powerfully with 5% higher probability for bootstrap subgroup classification^(26,27). However, these two reports asserted that the 287 bp typing region is useful to classify isolates into subtypes I to IV.

Totally, sequencing of the whole VP1 region or first part of the LTag is required to classify isolates into subgroup Ib-1 or Ib-2, supporting findings in previous reports^(14,27). Zheng and colleagues analyzed 30 typing regions and also full genome sequencing of BKVs derived out from different geographical areas of the world⁽¹⁹⁾. They reported that the phylogenetic analysis based on complete DNA sequences supports not only the subtype classification of BKV isolates, but also the sub-classification of subtype I isolates. In addition, this phylogenetic analysis allowed sub-classification of subtype IV into its subgroups. Also, they suggested that host-linked evolution is the general mode of polyomavirus (JC and BK viruses) evolution⁽¹⁹⁾. Additionally, their results indicated certain unique aspects of the relationship between BKV and humans. This was a theory for tracing the geographical origins of unidentified cadaver based on BK genotype in a report from Ikegaya and colleagues⁽²⁸⁾.

Zhong and colleagues⁽²⁰⁾ studied BKV genotypes of American, European, and Asian populations. They reported the highest frequency of subtype I in all populations compared to subtype IV which was variable among populations. Subgroup I/c was prevalent in native Japanese but was rare in the second generation of this population^(19,20). This observation can also be reconciled with the co-migration hypothesis. This hypothesis assumes that the children of migrant Japanese have acquired BKV infection not from their parents, but from European Americans living in their local community^(19,20). Therefore, it seems that prevalence of BKV subgroups among a geographical region might not be related to ethnicity.

By aligning the derived sequences with the reference sequence (accession number: JX195576), we found VP1 gene mutations which were different from other reports. All amino acid variations in this study (positions 60, 68, 72, 73 and 82) localize to the BC loop. On the other hand, subtypes are determined by the VP1 sequence between amino acids 61 and 83, which is the variable antigenic region and maps to the BC1 and BC2 loops.⁽²⁹⁾ Therefore, it was apparent that the variations were not randomly distributed but seemed to be arranged in "VP1 hotspots." Amino acids at positions 60, 68, 72, 73 and 82 in the VP1 protein showed an interesting pattern of changes⁽¹⁴⁾. Some researchers had suggested that amino acid substitutions at these five locations might result in type-determining changes in three-dimensional protein configurations⁽¹⁴⁾. Since the BC loop is believed to interact with the cellular receptor of BKV, it would be speculated that the genotype specific amino acid changes might alter BKV tissue tropism⁽¹⁴⁾. However, the finding of Pastrana and colleagues proved that different BKV genotypes have different cellular tropisms and pathogenic potentials in vivo⁽³⁰⁾.

Recently, two published data have pointed that like RNA, BKV viruses are able to produce quasispecies. A study had found that sequencing of BKV isolates subcloned from BKV in nephropathy patients revealed a high percentage of variants in the urine (40%) in the VP1 subtyping region⁽³¹⁾. In vitro analysis of several vi-

ral variants revealed that all variants which recovered from the urine of BKV associated nephropathy patients produced infectious viral particles. Studied BKVs were replication-competent in cell culture while some of the variants induced cytopathic changes in infected cells compared to the major BKV subtype and VP1 subtype I. These results suggest that rare BKV VP1 variants are more frequently associated with disease and that some variants could be more cytopathic than others in kidney transplant recipients⁽³¹⁾.

No phylogenetic analysis reports of BKV genome were found among patients with prostate cancers and benign prostatic hyperplasia tissues. In our study 93% of studied samples were derived out from patients with prostate cancers and benign prostatic hyperplasia tissues. This remarkable VP1 gene diversity of BKV may have an implication role for clinical diagnostics. BKV can cause malignancy in animal models. Still conclusive evidence is lacking regarding a causal connection between BKVs and human cancer⁽³⁰⁾.

Our study had some limitations. We used relatively few samples. Therefore, the results cannot be generalized to the Iranian population. Nevertheless, we consistently reported five new mutations in all studied patients which were not previously reported in Iranian patients which add to the value of our findings. Also as stated above we had no information regarding the serological tests of BKV infection in our patients.

CONCLUSIONS

Phylogenetic analysis of the VP1 gene sequences in our study supports the existence of genotypes 1b2 in the studied sample of Iranian patients in this study. Finding BKV subtype 1 in Iran was consistent with other Iranian studies, which had reported BKV among Iranian kidney transplanted patients. Our data documented the phylogenetic diversity of Iranian BKV and established the existence of clades not previously recognized in the literature. In this study BKV sequence of Iranian strains showed five mutations within the VP1 (positions 60, 68, 72, 73 and 82) which distinguished these strains from other type 1b2 sequences. Iranian BKV strains do not form distinct clusters from each other. However, they were classified as a unique cluster among 1b2 subtype sequences.

CONFLICT OF INTEREST

None declared.

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