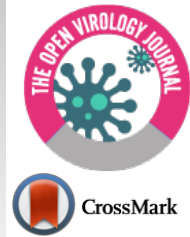




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## RESEARCH ARTICLE

### Molecular Characterization of BK Polyomavirus' Large T Antigen Gene Sequences Detected in Prostate Cancer Tissues of Sudanese Patients

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#### Abstract:

##### Background:

*BK virus*, which is associated with Prostate Cancer (PCa), have a global seroprevalence in humans. Based on the sequences of VP1 and the Large Antigen (LTA<sub>g</sub>) genes, there are four subtypes of BKV. Each subtype has its own subgroups.

##### Objective:

The aim of this study was to identify the BKV subtype that circulates among Sudanese patients with PCa.

##### Materials and Methods:

A total of 8 samples from our previous work on *BKV* were studied in this investigation. The LTA<sub>g</sub> gene was partially amplified (176nt) by a homemade PCR. All the amplicons were purified and subjected to sequencing. Bioedit version 7.0 and Mega X version 6.0 were used to analyze the sequence and compare the results with the BKV sequences and build a phylogenetic tree.

##### Results:

All the *BKV* LTA<sub>g</sub> gene sequences derived from Sudanese patients were classified with Subtype-1 *BKV* strains from Iran and Japan. Translated protein alignment showed that some isolates had identical amino acids with Iranian and Japanese strains, whereas others had a silent mutation. Interestingly, a point mutation was identified in the sequences of isolate 5 and 8 where adenine nucleotide (A) was replaced with Cytosine (C) at position 276, resulting in amino acid substitution.

##### Conclusion:

It was concluded that all the *BKV* isolates which circulated among Sudanese prostate tumor patients belonged to subtype 1. These findings only highlighted the need for the molecular detection and subtyping of *BKV* strains in Sudanese patients in order to better demonstrate the relationship between *BKV* infection and PCa.

**Keywords:** *BK virus*, Large T Antigen, PCR, Genotype, Prostate, Cancer, Benign prostatic hyperplasia, Point mutation.

#### Article History

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## 1. INTRODUCTION

The prostate gland is one of the most essential male accessory glands. This gland is susceptible to various pathological conditions, among which both malignant and benign conditions are the most common [1]. Benign Prostatic Hyperplasia (BPH), which is not a cancer, is common among older men and occurs when the prostate gland is enlarged.

When the gland becomes larger it ‘squeezes’ the urethra, thus causing several complications, such as difficulty urinating and frequent urination during the day [2]. PCa is a biologically heterogeneous tumor and is one of the leading causes of cancer death in men [3]. 241,740 new cases and 28,170 deaths were estimated in 2012 [4]. It is the second leading cause of cancer-related death in men in the United States [5]. The underlying causes of PCa are not fully understood, but it is likely to occur due to a combination of factors, such as aging, family history and dietary factors in addition to infectious agents [4 - 6]. Pooled data from population studies on PCa risk have reported

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an 80% increase in the risk of PCA in men with a history of prostatitis, although the detection of potential bias plays a role in it. Proliferative Inflammatory Atrophy (PIA) is common in normal and cancerous prostate and is a regenerative lesion after injury or trauma. It has been suggested that PIA is a precursor lesion of PCa and several genetic mutations have been identified in PIA, including glutathione S-transferase pi 1 (GSTP1) hypermethylation, p53 mutations, and alterations on chromosome 8 [7].

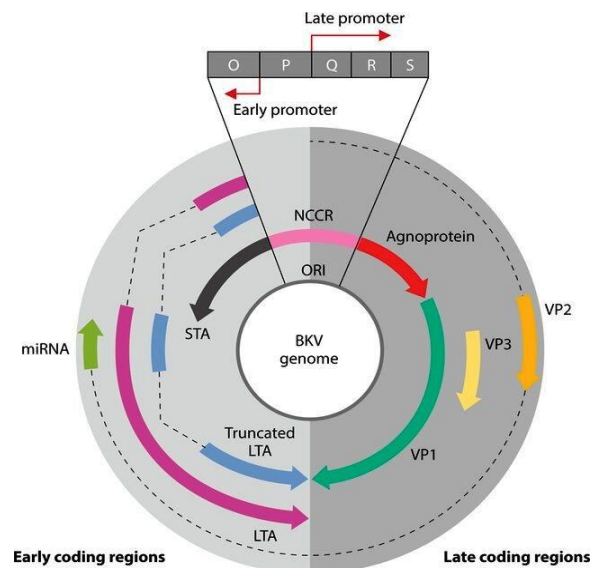
Several pieces of evidence support an infectious pathway in PCa development. In particular, Sexually Transmitted Infections (STIs) have been implicated in PCa etiology in many studies. A meta-analysis of 29 case-control studies found an increased relative risk of PCa in men with a history of any STI with an odds ratio of 1.5 and 95% Confidence Interval (CI), (1.3-1.7). Several sexually transmitted organisms have been detected in the prostate, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Treponema pallidum*, *human papillomavirus*, *herpes simplex virus*, and *human herpesvirus type 8*. Serum antibodies against *herpes simplex virus* and *Trichomonas vaginalis* in men have been associated with PCa risk, whereas the presence of the DNA of *human papillomavirus* in prostate tissues has also been associated with the risk of PCa [8]. Infectious agents may lead to cancer by several potential mechanisms. Although, direct cellular transformation can occur by viruses, several changes in the tissue microenvironment can occur if a chronic inflammatory state is reached after the infection. These include damage due to reactive oxygen species along with cytokine-induced angiogenesis and cellular proliferation, all of which may be involved in carcinogenesis [9].

The physiopathology of BKV-induced inflammation and the role of a cofactor in prostate and other cancers can be summarized by the fact that BKV T antigens, such as JCV, display multiple functions which alter the normal physiological metabolism of cells, ultimately leading to immortalization and neoplastic transformation. The main property of Tag in relation to the transformation and oncogenicity is its ability to bind and block the functions of tumor suppressor proteins p53 and pRB family (p105 RB1, p107 and p130 RB2). BKV Tag also induces numerical and structural chromosomal alterations in human embryonic fibroblasts, characterized by gaps, breaks, dicentric and ring chromosomes, deletions, duplications and translocations. Chromosome damage in human cells transfected with BKV early region was evident before the appearance of immortalization and the morphologically transformed phenotype [10 - 13].

The human *BK polyomavirus* is a member of the *polyomavirus* family. The virus has an icosahedral shaped capsid which is unenveloped, measuring about 40-50 nm. The capsid encloses a circular double-stranded DNA genome of approximately 5100 nucleotides coated by host-cell histones [14]. The virus was first isolated from the urine of a renal transplant patient in 1971 [14] and infects almost 90% of the human population worldwide. It rarely causes diseases in immune-competent individuals and resides in a subclinical persistent state in the urinary tract of healthy individuals and reactivates in immunosuppressed transplant patients, in whom

it is associated with certain diseases, such as hemorrhagic cystitis and *polyomavirus* nephropathy [15]. Urinary shedding has been reported to occur asymptotically and intermittently in healthy individuals [16].

The genome of *BKV* is divided into two transcriptional regions called early and late regions in addition to the third non-transcriptional region called the regulatory region. The genome of a typical *BK polyomavirus* codes for between 5 and 9 proteins, two from the early region and the rest from the late region. The early region is transcribed by the host cell's RNA polymerase II and codes for two proteins, the small and large tumor antigens, produced by alternative splicing [17]. The Large Tumor Antigen (LTag) is expressed early in the infectious cycle and is essential for virus proliferation. It is about 600-800 amino acids in length containing four well-conserved protein domains as well as several intrinsically disordered regions. The antigen has two primary functions, both related to virus replication. Its first function is to unwind the viral DNA and prepare it for replication, while the second is to destroy the cell cycle by interaction with the host cell protein. In addition, the LTag promotes cellular transformation by interfering with the tumor suppressor functions of Rb, p107, p130, and p53 (Fig. 1) [17 - 19]. Another protein that is expressed early in the *BKV* infectious cycle is the small Tumor Antigen (STAg). Unlike the LTag, it is not essential for viral replication, instead it induces tumorigenesis and promotes anchorage-independent growth of transformed cells by interaction with the cell protein phosphatase 2A (Fig. 1) [18, 19]



**Fig. (1).** Genome structure of *BKV*. The transcription of both early and late coding regions proceeds in a bidirectional way from the Origin of Replication (ORI) within the Noncoding Control Region (NCCR). The transcriptional splicing regions are represented by dashed lines. The late coding regions encode structural proteins (VP1, VP2, and VP3), while the early coding regions transcribe the tumorigenic proteins (LTA and STA). The late coding regions also encode the non-immunogenic Agno protein. The expression of miRNA complementary to the 3' end of LTA has been shown to be involved in replication control of *BKV* (36).

The first genotyping schema for *BKV* described by Jin *et al.* in 1993 was based on a very short segment of the VP1 gene (nucleotides 1744 to 1812). However, phylogenetic trees based on LTA sequences were introduced and validated recently [20]. The International Committee on Taxonomy of Viruses currently classifies *polyomaviruses* primarily according to the sequence identity of their LTag genes. This system has been questioned by phylogenetic studies suggesting that the evolutionary history of LTag and major capsid protein VP1 is divergent and that some modern *polyomaviruses* represent chimeric lineages [18, 19]. Based on the serological and genotyping method, the *BKV* isolated from various locations of the world is categorized into four subtypes [21]. Subtype I is the most dominant and has a worldwide distribution, followed by subtype IV which is mostly isolated from East Asia. However, subtypes II and III have low frequencies compared to the others [22]. In addition, the phylogenetic investigations revealed that there were four subgroups of subtype I, subgroups 1/a, 1/b-1, 1/b2 and 1/c. While, Subtype IV is divided into six subgroups, 4/a-1, 4/a-2, 4/b-1, 4/b-2, 4/c-1 and 4/c-2 [21].

In Sudan, the relationship between viruses and cancer has been studied in only a limited studies [23, 24]. In our previous study, the existence of the relationship between BKV and prostate cancer was determined [25] and the molecular characterization of BKV was described because only a few researchers have addressed *BKV* Subtypes. However, most previous studies have focused only on the isolates related to BKV nephropathy. The effect of each subtype on the clinical implications had previously been highlighted by various researchers [26, 27]. Some researchers have reported that the characterization of the genetic mutations of BK virus gene may have biological and clinical effects [28]. Thus, our study attempted to determine the phylogenetic characterization of *BKV* strains among Sudanese patients with a prostate disorder, such as prostate cancer and benign prostatic hyperplasia.

## 2. MATERIALS AND METHODS

### 2.1. Overall Design

A cross-sectional study was carried out based on the samples from our previous work on *BKV* to determine the phylogenetic characterization of *BKV* strains among Sudanese patients with a prostate disorder, such as prostate cancer and benign prostatic hyperplasia.

### 2.2. Study Population

A total of 8 samples from our previous work on *BKV* were studied in this investigation [25]. These samples were randomly selected from 21 positive samples. Samples 1 to 5 were obtained from PCa patients, while samples 6 and 7 were taken from BPH patients as well as one urine sample from a patient with BPH. All the patients were referred to central hospitals in Khartoum. Each test was done considering one sample as one patient.

### 2.3. Data Collection

An interviewer administered questionnaire was used to ask participants about their demographic, socioeconomic, and

geographical affiliation, cadmium contact, alcohol consumption, cancer grade along with detailed contact information for the communication of result and subsequent treatment if necessary.

### 2.4. Ethical Statement

Before conducting the study, the proposal of the study was ethically approved by the ethical committee of Omdurman Islamic University and the Ministry of Health. After that, informed consent from each patient and permission from the general managers of the hospitals were obtained.

### 2.5. Large T Antigen Gene as a Target for BKV Molecular Characterization

The first genotyping schema for *BKV* described by Jin *et al.* in 1993 was based on a very short segment of the VP1 gene (nucleotides 1744 to 1812). While phylogenetic trees based on the LTA sequences were introduced and validated recently [20]. The International Committee on Taxonomy of Viruses currently classifies *polyomaviruses* primarily according to the sequence identity of their LTag genes. This system has been questioned by phylogenetic studies suggesting that the evolutionary history of LTag and major capsid protein VP1 is divergent and that some modern *polyomaviruses* represent chimeric lineages [18, 19]. Therefore, the standard *BKV* subtyping and subgrouping was based on the phylogenetic analysis of 170 fragments within VP1 and Large T antigen genes (nucleotides 1564 to 2215 and 171 3021 to 3715, respectively) [29, 30].

However, in our study, only the Large T antigen gene amplification product (176 bp) is utilized in order to confirm the *BKV* isolates and to construct a simple phylogenetic tree that will give an idea about the subtypes which circulate in Sudanese patient with a prostate disorder, such as prostate cancer and benign prostatic hyperplasia. Unfortunately, subgroups of the *BKV* subtypes were not recognized in this study as the study was based only on the LTag gene to conduct the phylogenetic analysis. The *BKV* subgrouping will be a very interesting topic for future studies.

### 2.6. Viral DNA Extraction from Tissue Biopsy and Urine Specimens

About 25 mg of each tissue sample was incubated in lysis buffer (20 µl proteinase K (200 mg/ml) and 5µl of RNAase A). The DNA extraction was performed by the DNeasy® Tissue Kit (QIAGEN Company) according to the manufacturer's instructions and stored at -80 °C until further analysis. The quality of DNA was checked using the Nano-drop test. On the other hand, one ml of urine sample was incubated in lysis buffer (20 µl proteinase K (200 mg/ml) and 5µl of RNAase A). The DNA extraction was performed by the DNeasy® Urine Kit (QIAGEN Company) according to the manufacturer's instructions and stored at -80°C until further analysis.

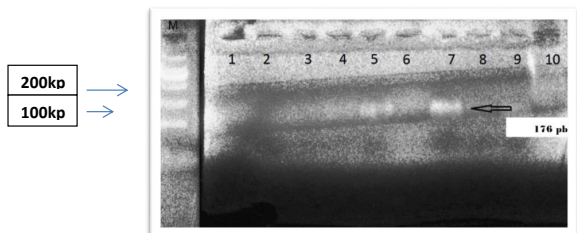
### 2.7. Target Large T Antigen Gene Amplification

*BK* viral early gene region (Large T antigen region 176bp) was amplified in a master mix reaction with a volume of 25µl containing 5 µl of DNA sample and 1µl of specific forward and reverse primer for primer F: 5'-AGTCTTTAGGGTCTT CTAC

C-3' and *BKV127-R*: 5'-GGTGCCAACCTATGGA ACAG-3' [30]. and was completed to 25µl with 13 µl distilled water. The amplification was done by using a TC-3000 conventional PCR Thermal Cycler (USA), completing 40 cycles of denaturation at 94°C for 1 min followed by initial denaturation at 94°C for 5 min, annealing at 55°C for 1 min, extinction at 72°C for 2 min and a final extension at the same temperature for 5 min. Finally, the conventional PCR products were subjected to gel electrophoresis (2% agarose gels) to visualize the band of the amplified target gene region with ethidium bromide (0.5 µg/mL) for 30 min in a UV-gel documentation system. The purified *BKV* genome of the Dunlop strain was used as a positive control, while distilled water was used as a negative control in each PCR run. The primer design and PCR protocol were implemented following the procedure used by Ibrahim *et al.* in 2016 [30].

**2.8. Purification of the DNA Product Before the Sequencing Process**

The success of automated sequencing critically depends on having a high purity template in the correct concentration. Therefore, the PCR products (Fig. 2) were purified by using a Gel Extraction Kit to remove all the unwanted elements from our template. This step was preceded by the detection of PCR template on a gel to confirm the presence of a specific product with an accurate amount. DNA purification was performed according to the standard guidelines of the Macrogen PCR product purification kit (supplied Macrogen, Inc. Korea).



**Fig. (2).** Conventional PCR Result. The products visualized by electrophoresis on ethidium bromide-stained 2% agarose gel. Lane M: low molecular weight marker DNA (100 bp MBI Fermentas) lane 1 contains the negative control, Lane 7 contains the *BKV* positive control. Lanes 2,3, 4 and 6 were negative samples, lane 5 shows a *BKV* positive band (176bp).

**2.9. Sequencing Process**

All the purified DNA amplicons (176 bp) (Fig. 2), including the positive control, were sent to Macrogen company (Macrogen, Inc. Korea) for direct sequencing with 10 pmol of

related PCR primers bi-directionally.

**2.10. Bioinformatics Analysis**

The obtained nucleotide sequences of large T antigen genes were assembled manually by the Finch TV software, and the cleaned sequences were searched for sequence similarity using nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Sequence Similarity Search - BLAST - *GenomeNet*. Highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using the BioEdit. Protein translation and modeling was done online by the CPH model server-3.2 and the EXPASSY translate tools, respectively. The phylogenetic tree was generated by a maximum likelihood method using the MEGA6 program from the aligned nucleotide sequences. The statistical robustness and reliability of the branching order within each phylogenetic tree were confirmed by applying bootstrap resampling (n = 1000 replicates). Sequences from different countries were retrieved from the gene bank and included in the generation of phylogenetic trees.

**2.11. Quality Control**

To exclude that point mutation is not an error generated during the sequencing process and to confirm the stability of the reagent, we included a purified *BKV* genome of the Dunlop strain as a positive control and distilled water as a negative control. The positive control yielded a sequence of nucleotides that were identical to that of the gene bank *BKV-DUN* (JO2038). Briefly, the amplification of beta-globin was done for all the samples to control DNA extraction. All the experiments were carried out in a contamination-free environment.

**3. RESULTS**

**3.1. Patients Description**

A total of 8 samples from our previous work on the *BKV* were studied in this investigation. Samples 1 to 5 were obtained from PCA patients, while samples 6 and 7 were taken from BPH patients as well as one urine sample from a patient with BPH. Patients were aged between 62 and 74 years with an average age of 68 ± 2.4 years. All the patients included were those who were referred to the central hospitals in Khartoum, three were from Central Sudan and two from the Western part. In addition, two patients were from the Northern parts and one from Southern Sudan. The histological identification Gleason scores for samples 1, 2, 3, 4 and 5 were 7, 6, 8, 7 and 8, respectively (Tables 1 and 2).

**Table 1. Mean, Std. Deviation, Maximum, and minimum age / years old for all patients.**

N	Valid	8 Age/ Years Old
	Missing	0
Mean		68.0000
Std. Deviation		4.40779
Minimum		62.00
Maximum		74.00

**Table 2. Patients' details description.**

Isolate No	Age	G.A*	P.T*	Cad*	Alcoh*	G.S*	Presence of Point Mutation (Transversion)	Presence of Silent Mutation
1	62	Central region	PCa*	No	No	7	-	-
2	66	Central region	PCa*	No	Yes	6	-	+
3	71	Northern region	PCa*	No	No	8	-	-
4	65	Western region	PCa*	No	Yes	7	-	-
5	73	Southern region	PCa*	No	Yes	8	+	-
6	74	Center region	BPH*	No	No	-	-	+
7	64	Northern region	BPH*	No	No	-	-	+
8	69	Central region	Urine*	Yes	No	-	+	-

G.A\* = Geographical affiliation P.T\* = patient type PCa\* = Prostate cancer BPH\* = benign prostatic hyperplasia, Cad\* = cadmium contact, Alcoh\* = Alcohol consumption, G.S\* = Gleason Score

**3.2. Blasting Result**

BLAST nucleotide search revealed that isolates 1, 3 and 4 had a 100% nucleotide identity with some of the band gaps with *BKV* isolates from Iran, Japan, Germany, USA and Zambia, having a GenBank accession number of GU130546.1, LC185075.1, NC\_025892, CAP-m9-USA-AY628233, and NC\_025896, respectively (Table 3). While the rest of the isolates had a 99% degree of similarity in the nucleotides with the USA isolate, which had a GenBank accession number of NC\_001538 (Table 4). Multiple sequence alignment of translated protein showed that isolates 1, 3 and 4 had identical amino acids with Iran, Japan, Germany, USA and Zambia, which had a GenBank accession number of GU130546.1,

LC185075.1, NC\_025892, CAP-m9-USA-AY628233 and NC\_025896, respectively (Tables 3 and 5). Moreover, the result identified a silent mutation within the nucleotide sequences of isolates 7, 6 and 2 at positions 262, 264 and 261, respectively. Once the type of mutation was silent, these isolates (2,6 and 7) were considered as identical amino acid sequences with the USA *BKV* isolate (GenBank accession number NC\_001538) (Tables 4 and 6). The most remarkable result that emerged from our data was the detection of a point transversion mutation in the sequences of isolates 5 and 8, where nucleotide adenine (A) was replaced by Cytosine (C) at position 276 which changed the codon AAC to ACC resulting in the replacement of amino acid Asparagine (N) by Threonine (T) at position 253 (Tables 4 and 6).

**Table 3. Multiple nucleotide sequence alignments. The alignments were performed using the Clustal W2 sequence alignment method through MEGA X program. The table showed the identical nucleotide sequence alignment between *BKV* isolates 1,3,4 of prostate cancer and isolates from different country in the world with the presence of some gaps.**

-	70												80												90											
Germanync025892	G	T	T	T	G	G	T	A	A	C	C	A	T	G	A	A	T	G	A	A	T	A														
cap m9us628233	.	C	.	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	G	.	.														
jpan lc185075.1	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
iran 130546.1	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
zambianc02586	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
isolate1	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
isolate3	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
isolate4	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														

**Table 4. Multiple nucleotide sequence alignments. The alignments were performed using the Clustal W2 sequence alignment method through MEGA X program. The table describe 99% nucleotides similarity between isolates 2,5,6,7,8 and USA isolate with some point mutations that either to be silent which can be exemplified by isolate 7,6 and 2 in position 262,264 and 261 respectively or transversion mutation in isolates 5,8 which reflect a mutation in position 276 that result in Amino acid Substitution.**

	261												270												280											
Usanc001538	T	G	T	T	C	C	A	C	C	A	C	T	G	A	C	A	A	T	G	A	T	G														
isolate8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.														
isolate7	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
isolate6	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.														
isolate5	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.														

(Table 6) contd....

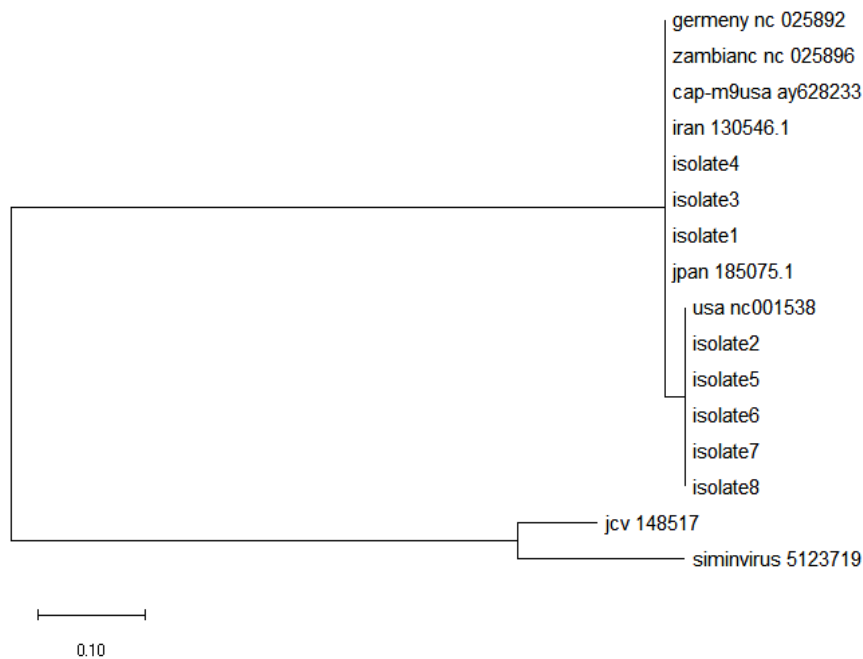
	261	'	'	'	'	'	'	'	'	'	'	270	'	'	'	'	'	'	'	280	'	'
isolate2	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

**Table 5. Multiple sequence amino acids alignments.** The alignments was done by using Clustal W2 sequence alignment method through MEGA X software. isolates 1,3 and 4 were showed an identical amino acids similarity with isolates from IRAN, JAPAN, GERMANY, USA and ZAMBIA with Gen Bank accession number of GU130546.1, LC185075.1, NC\_025892, CAP-m9-USA-AY628233 and NC\_025896 respectively.

Isolates	58	'	60	'	'	'	'	'	'	70	'	'	'	'	'	'	80				
germenync025892	Y	L	D	G	S	V	K	N	K	R	T	Q	I	F	P	V	E	D	P	K	D
cap m9usa628233	.	.	F	.	.	.	.	.	.	I	.	.	.	.	.	Y	.	.	.	.	.
jpan lc185075.1	.	.	.	S	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.
iran 130546.1	L	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.
zambianc 025896	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.
isolate1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
isolate3	*	*	L	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
isolate4	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

**Table 6. Multiple sequence amino acids alignments.** The alignments was done by using Clustal W2 sequence alignment method through MEGA X software. a transversion mutation was observed in position 253 for isolate 5 and 8 when compared to USA 001538 isolate sequences. While isolate 2, 6 and 7 have similar amino acids sequence similarity with USA 001538 isolate.

Isolates	170	'	'	'	'	245	'	'	'	'	250	'	'	'	'	255	'	
USAnc001538	Q	H	S	T	P	I	A	L	T	R	D	P	Y	N	N	L	D	S
Isolate2	*	*	*	*	*	*	.	.	.	.	.	.	*	*	*	*	*	*
Isolate7	*	*	*	*	*	*	.	.	.	.	.	.	*	*	*	*	*	*
Isolate 6	.	.	.	.	.	*	*	*	*	*	*	*	*	*	*	*	*	*
Isolate5	*	*	*	*	*	*	*	*	*	*	*	*	*	.	T	.	.	.
Isolate8	*	*	*	*	*	*	*	*	*	*	*	*	*	.	T	.	.	.



**Fig. (3).** Phylogenetic analysis of the BKV Large T antigen gene.

Another notable finding in our study was that *BKV* isolate 5 was obtained from the tissue sample of a PCa patient from Southern Sudan. While *BKV* isolate 8 was amplified from the urine sample of a BPH patient from Western Sudan. Interestingly, cadmium contact and alcohol consumption were observed for these patients, and it was found that the first patient did not have cadmium contact and did not consume alcohol, while the second was only in contact with cadmium (Table 2).

### 3.3. Phylogenetic Analysis Result

Phylogenetic analysis of the sequences recovered from the GenBank as well as the sequences obtained from the patient samples was performed. Complete sequences retrieved from the Gen Bank were aligned. A phylogenetic tree was then constructed in order to assemble all the 8 isolates according to the *BKV* subtypes and subgroups. The results showed that all the Sudanese *BKV* isolates belonged to the *BKV* Subtype-1 and were arranged into two subgroups; three isolates were combined with the same clade of GU130546.1, LC185075.1, NC\_025892, CAP-m9-USA-AY628233 and NC\_025896. While the rest of the isolates were arranged together with the same clade with the USA isolate Gen Bank accession number NC\_001538 (Fig. 3). These data reflected the distribution of *BKV* genotypes observed in the worldwide population, suggesting that this cohort of reference strains are relevant.

## 4. DISCUSSION

This study is not the first report on the molecular characterization of *BKV* in Sudan. However, most of the previous works have focused solely on the isolates associated with *BKV* nephropathy. Therefore, our study is the first of its kind to address the molecular characterization of *BKV* for PCa patients. Historically, *BKV* subtyping and subgrouping were based mainly on the phylogenetic analysis of some partial gene sequences (vp1 gene). While phylogenetic trees based on LTA sequences have been recently introduced and validated [20]. This study was based only on the large T antigen gene product 176 bp to construct the phylogenetic tree. All the sequences of the samples were analyzed using various bioinformatics tools in order to understand genotype variability, molecular epidemiology and evolutionary adaptability of circulating strains. The purpose for focusing on the LTA gene in our study was to determine the correlation between the *BKV* and prostate cancer in our previous work and to find a positive correlation between the presence of the virus and PCa and it is well known that LTA promotes cellular transformation by interfering with the tumor suppressor functions of p53 [4 - 6]. Thus, in this study, a point mutation was determined that could arise in this gene, resulting in antigen change. The latter event may affect the effectiveness of the antigen to bind with the antigen suppressing the tumor; this effect can be positive or negative.

In this study, there were two remarkable findings. Firstly, 3 out of 5 PCa *BKV* isolates were clustered into the same clade with Iranian and Japanese isolates. A previous study in Sudan revealed the *BKV* LTA sequences among kidney transplant recipients and reported that three of these isolates had nucleotide similarities with the Japanese isolates. However, their study differed from our study in the clinical source of the

isolates. They obtained their isolates from the urine samples of kidney transplant patients while our isolates were originated from the tissue samples of the patients with a prostate disorder. Moreover, in Africa, our isolates have similar nucleotide sequences with Zambian *BKV* isolate, while Ibrahim *et al.* found a nucleotide similarity between their isolate and the Ethiopian isolate [30]. Another significant point was that among our studied isolates, 5 *BKV* isolates (2 from PCa patients, 2 from BPH patients and 1 from the urine of BPH patients) were clustered together in the same clade with the USA strain, however, the similarity was 99%. Having 5 isolates in the same clade despite controlling the PCR condition and preventing DNA contamination can be considered as a strong evidence that the Sudanese isolates are likely to be closer to each other and could show genetic characteristics different from other strains found in the gene bank. Another point which may support our statement is the presence of point mutation which appears to be silent in some isolates, such as 2, 7 and 6 or transversion mutation like isolates 5 and 8. This latest finding may need further investigation considering the fact that both the mutant *BKV* isolates were from the hospitals in South and West Sudan which are closely related regions.

The histological identification Gleason scores for prostate cancer samples 1, 2, 3, 4 and 5 were 7, 6, 8, 7 and 8, respectively. Only isolate 5, which had a Gleason score of 8, showed a mismatched mutation, while other scores showed identical sequences upon alignment except for isolate 2 in which there was a silent mutation that had no significant impact on the translated amino acids. The association of the transversion mutation with the highest Gleason score in this study could raise a question whether the mutant variant of the *BKV* leads to a more aggressive type of PCa or not? In order to answer this question, further studies should be conducted by the inclusion of more *BKV* isolates from patients with prostate cancer grades.

Our result documented the circulation of one subtype (*BKV* Subtype 1) among Sudanese PCa and BPH patients with different age and geographical affiliations, regardless of the prostate Cancer Gleason scores. This finding was similar to that obtained by Maryam *et al.* in 2018 who concluded that all the *BKV* strains which were included in their study were classified under subtype 1. This is not surprising because most previous studies have revealed that despite different clinical samples, subtype I predominates in all the geographical regions of the world with higher frequencies, while subtype IV occurs at lower rates; and subtypes II and III occur rarely [31 - 36]. Unfortunately, as only the LTA gene was used to conduct the phylogenetic analysis, the *BKV* subtype 1 subgroups were not determined. The subgrouping of the *BKV* subtypes will be a very interesting topic for future studies.

In Sudan, there were no phylogenetic analysis reports on the *BKV* genome detected among patients with PCa and BPH tissues. In our study, more than 65% and 37% of studied samples were derived from PCa and BPH patients. Studies on the diversity of large T antigen gene sequences may have an implicit role in understanding the development of PCa by *BKV* because the LTA gene, together with a small T antigen gene,

produces antigens that are responsible for virus carcinogenesis [18, 19]. Our study had some limitations. Due to a lack of funding, only LTA<sub>g</sub> gene sequences were used in this study. A few numbers of isolates were included in this study. Therefore, the results may not be generalized. Nevertheless, two new transversion mutations among all the studied patients were consistently reported. This latest finding has not been reported before among Sudanese patients, which increases the value of our findings.

## CONCLUSION

The results of this study emphasized the importance of early detection and characterization of newly emerging genotypes. It was concluded that all the *BKV* isolates which circulate among Sudanese prostate tumoral patients belonged to subtype 1, and two isolates showed transversion mutations within the large T antigen amino acids sequence (positions 253), in addition to the presence of a silent mutation in some other isolates. These findings highlighted the need for the molecular detection and typing of *BKV* in Sudan for a better demonstration of the relationship between the virus and PCa. Moreover, our sequence analysis was done on the Large T antigen gene rather than the VP1 gene which is used as a standard target for the determination of *BKV* subtypes. Therefore, further studies must be conducted on molecular genotyping of *BKV* in Sudan by studying phylogeny through the amplification of both the above-mentioned genes with the inclusion of more *BKV* isolates, focusing on prostate cancer grades and patient age in addition to their geographical affiliations as well as the performance of a cell culture, transfection and *BKV* rescue to demonstrate that DNA *BKV* belonged to initially replicating *BKV* viruses. These may enable a better demonstration of the correlation between the *BKV* isolates and PCa.

## LIST OF ABBREVIATIONS

<b>PCa</b>	=	Prostate Cancer
<b>BPH</b>	=	Benign Prostatic Hyperplasia
<b>LTA<sub>g</sub></b>	=	Large Tumor Antigen
<b><i>BKV</i></b>	=	BK Virus
<b>PCR</b>	=	Polemrse Chain Reaction

## AUTHORS' CONTRIBUTION

BG and MO performed the main experiments, BG and MO collected patients' samples and information. BG and IS designed the experiments and wrote the manuscript. All the authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was ethically approved by the ethical committee of Omdurman Islamic University and the Ministry of Health under reference no. 207MLS/2017, Sudan.

## HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the guidelines of

the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects 1964.

## CONSENT FOR PUBLICATION

Informed consent from each patient and permission from the general managers of the hospitals were obtained.

## AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author (Babbiker M.T. Gorish) on reasonable request.

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None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## REFERENCES

- [1] Verhamme KM, Dieleman JP, Bleumink GS, *et al.* Triumph Pan European Expert Panel. Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic hyperplasia in primary care--the Triumph project. *Eur Urol* 2002; 42(4): 323-8. [[http://dx.doi.org/10.1016/S0302-2838\(02\)00354-8](http://dx.doi.org/10.1016/S0302-2838(02)00354-8)] [PMID: 12361895]
- [2] Grossman DC, Curry SJ, Owens DK, *et al.* US Preventive Services Task Force. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2018; 319(18): 1901-13. [<http://dx.doi.org/10.1001/jama.2018.3710>] [PMID: 29801017]
- [3] Rodriguez H, Levican J, Muñoz JP, *et al.* Viral infections in prostate carcinomas in Chilean patients. *Infect Agent Cancer* 2015; 10: 27. [<http://dx.doi.org/10.1186/s13027-015-0024-y>] [PMID: 26330890]
- [4] Delbue S, Matei DV, Carloni C, *et al.* Evidence supporting the association of polyomavirus BK genome with prostate cancer. *Med Microbiol Immunol (Berl)* 2013; 202(6): 425-30. [<http://dx.doi.org/10.1007/s00430-013-0304-3>] [PMID: 23821367]
- [5] Elimam ME, Abdelraof S. Incidence of carcinoma of the prostate in patients with normal prostatic specific antigen following prostatectomy. *Glob J Medi Res* 2013; 13: 26-35.
- [6] Provenzano M, Keller P. The potential therapeutic usefulness of targeting BK polyoma virus in prostate cancer. *Chemo Open Access* 2015; 5: 1.
- [7] Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, *et al.* Identification of viral infections in the prostate and evaluation of their association with cancer. *BMC Cancer* 2010; 10: 326. [<http://dx.doi.org/10.1186/1471-2407-10-326>] [PMID: 20576103]
- [8] Wright JL, Lin DW, Stanford JL. Circumcision and the risk of prostate cancer. *Cancer* 2012; 118(18): 4437-43. [<http://dx.doi.org/10.1002/cncr.26653>] [PMID: 22411189]
- [9] De Marzo AM, Platz EA, Sutcliffe S, *et al.* Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 2007; 7(4): 256-69.



- [10] Das D, Wojno K, Imperiale MJ. BK virus as a cofactor in the etiology of prostate cancer in its early stages. *J Virol* 2008; 82(6): 2705-14. [http://dx.doi.org/10.1128/JVI.02461-07] [PMID: 18160432]
- [11] Sina A. Detection of *Polyomaviral* large T antigen in benign prostatic hyperplasia and prostate carcinoma tissues. *JKIMSU* 2015; 4: 26-33.
- [12] Monini P, Rotola A, Di Luca D, *et al.* DNA rearrangements impairing *BK virus* productive infection in urinary tract tumors. *Virology* 1995; 214(1): 273-9. [http://dx.doi.org/10.1006/viro.1995.9928] [PMID: 8525628]
- [13] Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP. Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. *Prostate* 2002; 53(4): 263-76. [http://dx.doi.org/10.1002/pros.10157] [PMID: 12430138]
- [14] Gardner SD, Field AM, Coleman DV, Hulme B. New human *papovavirus* (*B.K.*) isolated from urine after renal transplantation. *Lancet* 1971; 1(7712): 1253-7. [http://dx.doi.org/10.1016/S0140-6736(71)91776-4] [PMID: 4104714]
- [15] Tognon M, Corallini A, Martini F, Negrini M, Barbanti-Brodano G. Oncogenic transformation by *BK virus* and association with human tumors. *Oncogene* 2003; 22(33): 5192-200. [http://dx.doi.org/10.1038/sj.onc.1206550] [PMID: 12910256]
- [16] Dalianis T, Hirsch HH. Human *polyomaviruses* in disease and cancer. *Virology* 2013; 437(2): 63-72. [http://dx.doi.org/10.1016/j.virol.2012.12.015] [PMID: 23357733]
- [17] Ahuja D, Sáenz-Robles MT, Pipas JM. *SV40* large T antigen targets multiple cellular pathways to elicit cellular transformation. *Oncogene* 2005; 24(52): 7729-45. [http://dx.doi.org/10.1038/sj.onc.1209046] [PMID: 16299533]
- [18] Yu J, Boyapati A, Rundell K. Critical role for *SV40* small-t antigen in human cell transformation. *Virology* 2001; 290(2): 192-8. [http://dx.doi.org/10.1006/viro.2001.1204] [PMID: 11883184]
- [19] Maurizio P, Etienne XK. The potential therapeutic usefulness of targeting *BK Polyomavirus* in prostate cancer. *Chemo Open Access* 2015; 5: 1-2.
- [20] Luo C, Bueno M, Kant J, Martinson J, Randhawa P. Genotyping schemes for polyomavirus BK, using gene-specific phylogenetic trees and single nucleotide polymorphism analysis. *J Virol* 2009; 83(5): 2285-97. [http://dx.doi.org/10.1128/JVI.02180-08] [PMID: 19109389]
- [21] Kaydani GA, Makvandi M, Samarbazfzadeh A, *et al.* Prevalence and distribution of *BK virus* subtypes in renal transplant recipients referred to golestan hospital in ahvaz, Iran. *Jundishapur J Microbiol* 2015; 21(8): e16738.
- [22] Andi K, Olaf R, Bininda E, Peter W, Roland Z. Evolution of four *BK virus* subtypes, infection, genetics and evolution. *Infect Genet Evol* 2008; 8: 632-43. [http://dx.doi.org/10.1016/j.meegid.2008.05.006]
- [23] Gorish BM. Frequency of HBV among hepatocellular carcinoma patients in khartoum state, sudan. *J Carcinog Mutagen* 2018; 9: 320. [http://dx.doi.org/10.1038/nrc2090] [PMID: 17384581]
- [24] Dafalla AB, Elnil YF, Gorish BM. Seroprevalence of cytomegalovirus infection among leukemic patients in khartoum state. *Virology* 2018; 7: 183.
- [25] Gorish BMT, Ournasseir MEH, Shammat IM. A correlation study of *BK Polyoma Virus* infection and prostate cancer among sudanese patients - immunofluorescence and molecular based case-control study. *Infect Agent Cancer* 2019; 14: 25. [http://dx.doi.org/10.1186/s13027-019-0244-7] [PMID: 31548852]
- [26] Motazakker M, Bagheri M, Imani M. Subtyping of *BK Virus* in iranian turkish renal transplant recipients by RFLP-PCR. *Maedica (Buchar)* 2012; 7(1): 10-3. [PMID: 23118813]
- [27] Wang ZY, Hong WL, Zhu ZH, *et al.* Phylogenetic reconstruction and polymorphism analysis of *BK virus* VP2 gene isolated from renal transplant recipients in China. *Exp Ther Med* 2015; 10(5): 1759-67. [http://dx.doi.org/10.3892/etm.2015.2723] [PMID: 26640547]
- [28] Sharma PM, Gupta G, Vats A, Shapiro R, Randhawa P. Phylogenetic analysis of polyomavirus BK sequences. *J Virol* 2006; 80(18): 8869-79. [http://dx.doi.org/10.1128/JVI.00510-06] [PMID: 16940499]
- [29] Descamps V, Martin E, Morel V, *et al.* Comparative evaluation of three nucleic acid-based assays for *BK virus* quantification. *J Clin Microbiol* 2015; 53(12): 3822-7. [http://dx.doi.org/10.1128/JCM.02116-15] [PMID: 26424842]
- [30] Ibrahim AH, Hisham NA, Yousif FH, *et al.* Molecular characterization of polyomaviruses (*BKV*, *JCV*) in a symptomatic kidney transplant recipients in sudan. *AJIDM* 2016; 4: 44-51.
- [31] Chehadeh W, Kurien SS, Nampoory MR. Molecular characterization of BK and JC viruses circulating among potential kidney donors in kuwait. *BioMed Res Int* 2013; 2013683464 [http://dx.doi.org/10.1155/2013/683464] [PMID: 23936831]
- [32] Vaezjalali M, Azimi H, Hosseini SM, Taghavi A, Goudarzi H. Different strains of *BK polyomavirus*: VP1 sequences in a group of iranian prostate cancer patients. *Urol J* 2018; 15(2): 44-8. [PMID: 29277885]
- [33] Krumbholz A, Zell R, Egerer R, *et al.* Prevalence of *BK virus* subtype I in Germany. *J Med Virol* 2006; 78(12): 1588-98. [http://dx.doi.org/10.1002/jmv.20743] [PMID: 17063524]
- [34] Jin L, Gibson PE, Knowles WA, Clewley JP. *BK virus* antigenic variants: Sequence analysis within the capsid VP1 epitope. *J Med Virol* 1993; 39(1): 50-6. [http://dx.doi.org/10.1002/jmv.1890390110] [PMID: 7678637]
- [35] Agostini HT, Brubaker GR, Shao J, *et al.* *BK virus* and a new type of *JC virus* excreted by HIV-1 positive patients in rural Tanzania. *Arch Virol* 1995; 140(11): 1919-34. [http://dx.doi.org/10.1007/BF01322682] [PMID: 7503691]
- [36] Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R. *BK polyomavirus*: clinical aspects, immune regulation, and emerging therapies. *Clin Microbiol Rev* 2017; 30(2): 503-28. [http://dx.doi.org/10.1128/CMR.00074-16] [PMID: 28298471]