RESEARCH ARTICLE

Editorial Process: Submission:06/06/2023 Acceptance:03/05/2024

Prevalence of BK and JC Polyomaviruses among Patients with Breast Cancer

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Abstract

Introduction: Breast cancer, a pervasive invasive carcinoma among women globally, afflicts approximately 12% of women worldwide. Previous studies have indicated that certain viruses, including oncogenic viruses such as polyomaviruses BK and JC, may play a role in the development of breast cancer. In light of this, the present study endeavors to assess the incidence of BKV and JCV virus in breast cancer patients. **Materials and Methods:** One hundred formalin-fixed paraffin-embedded tissue samples were procured and subjected to deparaffinize by xylene, followed by DNA extraction through the phenol-chloroform methodology. Detection and genotyping of BKV and JCV were carried out utilizing specific primers via PCR analysis. **Results:** Merely 2 out of 100 (2%) ductal carcinoma in situ with grade 2 specimens exhibited positivity for BK virus genotype IV, whereas JC virus DNA was not discerned across all the samples. **Discussion:** The findings of the current investigation demonstrate that there was an absence of JC virus detection in the breast biopsy. Additionally, a small fraction of patients diagnosed with ductal carcinoma exhibited a low prevalence of genotype IV polyomavirus BK at a rate of 2%. However, in order to gain a more comprehensive understanding of the incidence of BKV and JCV in breast cancer, a substantial number of breast samples must undergo investigation.

Keywords: Polyomavirus BK- Polyomavirus JC- Breast cancer- ductal carcinoma

Asian Pac J Cancer Prev, **25 (3)**, 821-827

Introduction

Breast cancer is a pervasive form of invasive cancer that has a global impact on women, with approximately 2.3 million new cases and 685,000 deaths reported in 2020 [1]. The incidence and mortality rates of breast cancer vary among countries, with Belgium having the highest age-standardized incidence rate of 112.3 per 100,000 population, and Iran having the lowest rate of 35.8 per 100,000 population [2]. Furthermore, Fiji had the highest age-standardized mortality rate of 41.0 per 100,000 population, whereas South Korea reported the lowest rate of 6.4 per 100,000 population [3].

Multiple factors including environmental and genetic factors, population structure, lifestyle choices, smoking, and alcohol consumption have been identified as potential risk factors for the development of breast cancer (Allahqoli et al., 2022; Momenimovahed, 2019) [2] [4]. Various viruses including human endogenous retroviruses (HERVs), human mammary tumor virus (HMTV), Torque teno virus (TTV), Polyomavirus BK (BKV), and Polyomavirus JC (JCV) have been linked with breast cancer in multiple studies (McLaughlin-Drubin, 2008; Hachana et al., 2012; Dehcheshmeh et al., 2020) [5-7]

The BK virus, also known as Human polyomavirus 1 is primarily transmitted via the inhalation or fecal-oral route during childhood [8, 9] and after primary infection in mononuclear blood cells and urinary tract kidneys.

Human polyomavirus 1 (BK virus) remains latent and excretes the virus in the urine, resulting in asymptomatic infection. A significant proportion (80% to 90%) of the global population has shown IgG positivity against BK virus [10]. Reactivation of latent BK virus can occur in immunosuppressed individuals with HIV infection or kidney transplant recipients, resulting in the development of BKV-associated nephropathy and an increased risk of allograft loss [11-13]. It is noteworthy that patients presenting with unexplained encephalopathy and compromised immune systems have demonstrated reactivation of the BK virus, as reported by Darbinyan et al. (2016) [14]. Similarly, the development of hemorrhagic cystitis in Hematopoietic Cell Transplantation (HCT) patients is attributed to Polyomavirus BK infection, as indicated by Aldiwani et al. (2019) [15].

Polyomavirus JC also known as Human polyomavirus 2 has been identified as the etiological agent responsible

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for a rare fetal disease called progressive multifocal leukoencephalopathy (PML) that occurs under conditions of immunosuppression, according to Ferenczy et al. (2012) [16]. Comar et al (2012) [17] and Polo et al (2004) [18] have reported multiple transmission routes for the JC virus, including fecal-oral, urogenital, respiratory, perinatal, and transplacental modes. The sequencing of the VP1 region of JC virus has led to the identification of eight JC virus genotypes, which are distributed across various regions of the world [7].

The large T antigen expressed by both polyomavirus JC virus and BK virus has been found to bind to the p53 and pRB families (p105, RB1, p107, p130) of cells, which leads to an increase in cell proliferation, transformation, and the development of cancer [19, 20]. Furthermore, the JC and BK viruses' genomes exhibit the ability to integrate with the host genome, leading to genetic instability and ultimately resulting in the formation of malignant cells [21, 22].

Both JC and BK viruses have also been identified in certain cases of breast carcinomas; however, the significance of these observations with respect to a potential causal relationship between these polyomaviruses and breast cancer requires further investigation [6].

JC virus has been associated with various types of cancers, including glioblastoma, medulloblastoma, oligodendroglioma, primary CNS CNS lymphoma, breast cancer, and colorectal cancer [21] [23, 24]. The BK virus has been found to play a role in various forms of cancer, including breast cancer, brain cancer, meningioma, osteosarcoma, bladder cancer, kidney cancer, hepatoblastoma, prostate cancer, cervix cancer, pancreatic islets cancer, colon rectum cancer, kaposi's sarcoma, and lymphoma [25, 26].

Considering the oncogenic role of BK and JC viruses, this study was conducted to investigate the detection of BKV and JCV DNA in patient samples with breast cancer in Ahvaz city, the capital of Khozestan province located in the southwest region of Iran.

Materials and Methods

Sample collection and DNA extraction

During the period of 2018 to 2019, one hundred formalin-fixed paraffin-embedded tissue samples were acquired, inclusive of 70 instances of invasive ductal cancer, 25 instances of in situ ductal cancer, 3 instances of mucinous cancer, one instance of invasive lobular cancer, one instance of medullary cancer, and 3 instances from Imam Khomeini Hospital in Ahvaz city. The diagnosis of cancer was verified by a pathologist. The criteria for inclusion were the confirmation of samples that tested positive for breast malignancy tumors, while the criteria for exclusion was the inclusion of non-malignant tumor samples, specifically fibroadenomas.

Deparaffinization was executed utilizing xylene and ethanol (Merk, Germany). In the initial step, the specimens were placed in microtubes, followed by the addition of xylene, and kept at a temperature of 45°C for 15 minutes. The mixture was subjected to centrifugation at a rate of 14000rpm. This process was repeated once more. Subsequently, the supernatant was eliminated, and 1 ml of absolute ethanol was introduced to facilitate precipitation. The resulting solution was stored at room temperature for 10 minutes and then centrifuged again at 14000rpm for a duration of 1 minute. The supernatant that was discarded underwent a repetitive procedure where 70% ethanol was introduced under similar conditions. Finally, the supernatant was discarded again and all microtubes were exposed to a temperature of 65°C for 5 minutes to achieve evaporation of the residual ethanol. The resulting pellet was then used for DNA extraction [23]. Evaluation of the purity and concentration of the extracted DNA was conducted utilizing a Nanodrop device.

Human polyomavirus 2 (JC virus) detection

To determine the genotyping of JC virus, specific primers were utilized for the amplification of the VP1 region, as shown in Table 1. The PCR reaction mixture was prepared by adding 12 µl master (Sinaclon Iran), 0.5 µl (µM) of both forward and reverse primers, 5 µl of 300 ng isolated DNA sample as a template, and distilled water up to 25 µl. The reaction mixture was then subjected to the PCR machine (PeqLab, Germany) under the following thermal conditions and cycling program: denaturation at 94 °C for 5 minutes, followed by 37 cycles of a three-step PCR program consisting of denaturation at 94°C for 45 seconds, annealing at 59°C for 50 seconds, elongation at 72 °C for 50 seconds, and final extension at 72°C for 10 minutes. Positive results were indicated by the appearance of a PCR product of 215 bp on the gel electrophoresis [27].

Detection of Human polyomavirus 1 (BK virus 1) (LTAg)

The detection of BK virus LTAg was initiated by performing semi-nested PCR. In the first PCR run, a master mix PCR (Sinaclon, Iran) was utilized, consisting of 12µl master mix (Sinaclon, Iran), 0.5µl (10µM) of each forward (BKJC Out-1: 5'-AAGTCTTTAGGGTCTTCTAC-3') and reverse primers (BKJC Out-2: 5'- GTGCCA ACCTATGGAACAGA-3'), 7 µl of 400 ng of isolated DNA sample as a template, and distilled water up to 25 µl. The PCR master mix was then subjected to a thermal cycler (PeqLab Biotechnologie –Germany) with the following program: 1 cycle of 94ºC for four minutes, followed by 40 cycles of 94ºC for one minute, 55ºC for one minute, and 72ºC for 1.5 minutes. The final extension was at 72ºC for six minutes. The PCR product was electrophoresis onto a 2% w/v agarose gel, and the size of the first round of PCR was 176 bp, indicating a positive test. For the second PCR, 1µl of the first run product was used as a template, and the primers used were BKJC Out-1, 5'-AAGTCTTTAGGGTCTTCTAC-3', and BKIN: 5'- GAGTCCTGGTGGAGTTCC-3', with the same quantity of master mix and thermal cycling condition. The anticipated semi-nested-PCR products was the generation of fragments 176 bp and 149 bp respectively [28].

Detection of BK virus(VP1)

The detection of the BK virus genome was performed via PCR utilizing specific primers (Table 1) to detect the partial region of the VP1. The PCR reaction mixture consisted of 12 µL master mix (Sinaclon,Iran), 0.5 µL (10

μM) each of forward and reverse primers, 7 μL of 300 ng of isolated DNA sample as a template, and distilled water up to 25 μL, subsequently subjected to the PCR machine (PegLab) with the following cycling program: initiation, denaturation at 94°C for 5 minutes, followed by 37 cycles under thermal conditions of denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes [29]. In the second run, denaturation was performed at 94°C for 5 minutes, followed by 30 cycles under thermal conditions of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. The PCR products were electrophoresed in agarose gel and the presence of 327 bp indicated a positive result [29].

Sequencing and Phylogenic Analyses

The BK virus PCR product that tested positive underwent sequencing by Applied Biosystem 3500 instrument, USA. The sequencing procedure was conducted in both the forward and reverse directions. To determine the BK virus genotyping, the results from the partial sequencing of the VP1 region of two BK virus isolates were aligned with the NCBI BK virus database (https://blast.ncbi.nlm.nih.gov). The sequencing outcome was submitted to GenBank to obtain the accession number. The evaluation of the two VP1 sequence of isolates BK virus was subjected to MEGA software version 6 using the Maximum likelihood method under the Tamura-Nei model and 1000 bootstrap replicates to construct a phylogenetic tree (Figure 1).

Statistical analysis of the data

The study involved statistical analysis of the data. SPSS version 21 software was used to determine the ages of the subjects using mean and standard deviation techniques. The incidence of BK virus and JC virus in samples of breast cancer and cancer grades was evaluated using the chi-square test (x^2). A P-value of less than 0.05 was considered statistically significant.

Results

Demographic and pathological results

The breast cancer specimens consisted entirely of female patients, with the range of ages spanning from 29 to 84 years and a mean age of 52.81 years. In terms of histological subtype, the majority of samples (70%) were invasive ductal carcinomas, with in situ ductal carcinoma accounting for 25% of cases. Additionally, there were 3 cases (3%) of mucinous cancer, 1 case (1%) of invasive lobular cancer, and 1 case (1%) of medullary cancer.

In Table 2, a mere 2 (2%) instances of BK virus were

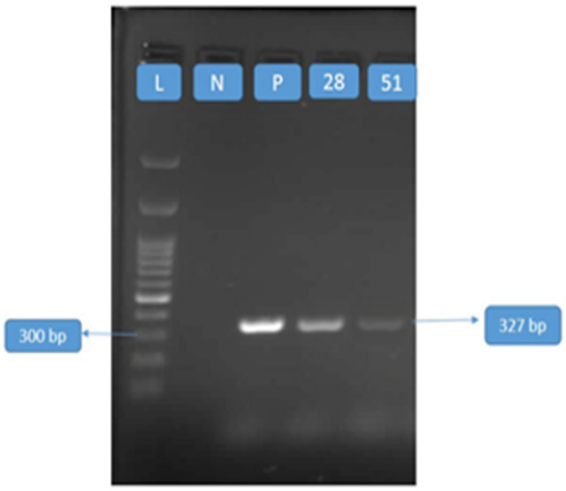

Figure 1. Displays the Detection of Positive BK Virus through the Application of PCR Analysis. Utilizing a 100bp size molecular marker, a negative control, a positive control, sample number 28, and sample number 51, PCR analysis detected positive BK virus.

Table 1. Primers for BK Virus and JC Virus VP1

Primer	Sequence	Pruduct size	Reference
JCV P1 F	5′-ACAGTGTGGCCAGAATTCACTACC 3′	215 bp	(Jin, 1993) [27]
JCV VP1 R	1924 -1902, 5′-TAAAGCCTCCCCCCCAACAGAAA-3′	215 bp	(Jin, 1993)
BKV VP1 F Outer	5′-ATCAAAGAACTGCTCCTCAAT-3′(nucleotides 1480-1500)	579 bp	(Pietropaolo, 1998) [29]
BKV VP1 R Outer	5′-GCACTCCCTGCATTTCCAAGGG-3′(nucleotides 2038-2059)	579 bp	((Pietropaolo, 1998) [29]
BKV VP1 F Inner	5′-CAAGTGCCAAAACTACTAAT-3′(nucleotides 1630-1649)	327 bp	(Jin,1993b) [30]
BKV VP1 R Inner	5′-TGCATGAAGGTTAAGCATGC-3′(nucleotides 1956-1937)	327 bp	(Jin,1993b) [30]

Table 2. Frequency of BK Virus in Breast Cancers

Type of Cancer	GradeI		GradeII		GradeIII		GradeIV		Total	P value
	BKV +ve/-ve		BKV +ve/-ve		BKV +ve/-ve		BKV +ve/-ve			
Invasive ductal carcinoma	17	-ve	38	-ve	15	-ve	_	-ve	70	
Ductal carcinoma in situ	4	-ve	2/12(2%)	+ve	6	-ve	3	-ve	25	0.11
Mucinous carcinom	1	-ve	2	-ve	_	_	_	_	3	
Invasive lobular carcinom	1	- ve	_	_	_	_	_	_	1	
Medullary carcinoma	-		-		1	-ve	-		1	
Total	23		52		22		3		100	

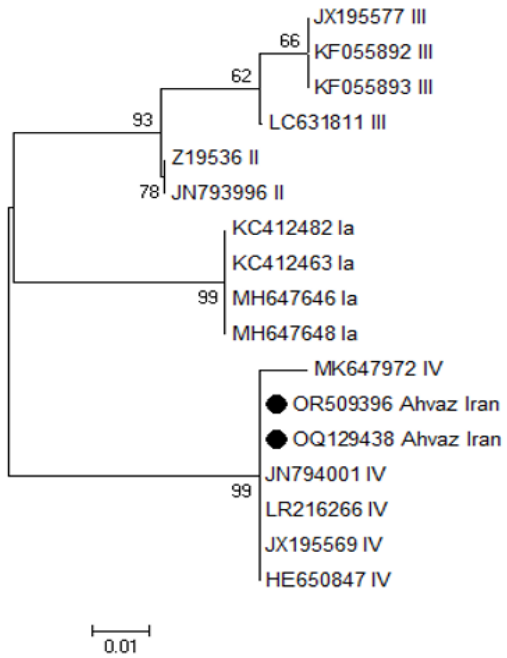

Figure 2. A Maximum Likelihood Approach was Utilized to Construct a Phylogenetic Tree, Denoted as Figure 2 A.The phylogenetic tree results indicated that the BKV isolated from Ahvaz (OQ129438 and OR509396) clustered with BK viruses genotype IV, specifically JN794001.1 and MK647972.1 which were isolated in France. The Tamura-Nei model was employed for the Maximum Likelihood method with 1000 bootstrap replicates. Furthermore, the scale bar was set at 002.

detected in ductal carcinoma in situ with grade 2, however, the prevalence of BK virus in breast cancers was not deemed statistically significant ($p<0.11$).

PCR for detection of JCV and BKV

The findings of the PCR test conducted on 100 breast cancer samples revealed the presence of 2 cases, constituting 2% of the total, which presented grade 2 ductal carcinoma in situ. These cases tested positive for BK virus genome in two separate tests of LTAg and Vp1 (Photograph 1). Notably, all breast tissue samples tested negative for JC virus genome. The age range of patients who tested positive for BK virus was between 48 and 62 years. Upon performing VP1 sequencing of the two BK virus positive cases, it was found that there was a 100% molecular conformity with BK virus genotype IV (JN794001.1, MK647972.1) that had previously been isolated in France.

Discussion

Breast cancer, constituting 12% of invasive cancers among women, is a pervasive health challenge, and ranks as the second most common cause of death globally [31, 32]. The development of breast cancer is influenced by various risk factors such as early onset of menstruation, late or no pregnancy, menopause beyond the age of 55 years, consumption of oral contraceptives, alcohol intake, family history of breast cancer, and smoking [33].

In the present investigation, all samples of breast cancer tissue exhibited negative results for the presence of JC virus genome. In Iran, Dowran and colleagues conducted a study encompassing a total of 300 breast biopsy tissue samples, comprising 150 malignant and 150 benign samples, and yielded negative findings for both JC virus and BK virus [34] . On the other hand, Hachana and associates reported that 23% (28/123) of breast cancer tissues were found to be infected with JCV [6]. It is important to note that the reactivation of JC virus in individuals with immunosuppression leads to fatal lytic destruction of oligodendrocytes, which is commonly known as progressive multifocal leukoencephalopathy (PML) disease [16].

During the course of our investigation, it was discovered that a mere 2% of the subjects' samples exhibited positivity for the BK virus genome. Two PCR assays were employed to confirm the presence of this genome. One of these assays was solely designed for the detection of Large T Antigen while the other assay, VP1 BK virus detection, was utilized to determine the genotyping of the BK virus. Notably, the positive BK virus was found in patients diagnosed with ductal carcinoma. Further, the detected BK virus exhibited genotype IV and was clustered with BK viruses genotype IV (JN794001.1, MK647972.1) that were isolated from France (Figure 2).

It is noteworthy that no detection of BK virus in breast cancer has been reported in Iran thus far. Dowran et al reported no detection of BK virus in patients with breast cancer [34]. Another study carried out in Australia demonstrated that all 54 breast cancer samples were negative for BKV DNA by Real-time PCR [35].

In our study, the genotype detected for BKV was IV. It has been reported that the dominant variant for BKPyV in eastern Asia is genotype IV, as found by Chen et al. The frequency of BK Virus genotype IV was observed to be 65% in north-western China, 88% in south-western China, 53% in Vietnam, and 100% in Mongolia, while the prevalence of BKPyV IV was low, at 8%, in Japan, according to Chen et al, 2006 [36].

However, as of yet, there is no definitive-approved treatment available for BK virus-associated Hemorrhagic cystitis (BKV-HC) in patients after Hematopoietic stem cell transplantation (HSCT). The limitation of this study was large sample size is required to explore for association of BK virus and JC virus with breast cancer, second the role of other Oncogenic Viruses Breast Cancer including Mouse Mammary Tumor Virus (MMTV), Bovine Leukemia Virus (BLV), Human Papilloma Virus (HPV), and Epstein-Barr Virus (EBV) have been investigated [37] which have not been investigated in this study. JCV remains latent in the kidneys, lymph nodes, bone marrow tonsil and brain of healthy and immunosuppressed individuals without PML (Randhawa et al,2005; Atwood et al,1992; Tan et al,2009; Monaco et al,1998; Elsner ,1992) [38-42].

The detection of BK virus has been observed in the urine, lymphocytes, and peripheral blood mononuclear cells (PBMCs) of both healthy subjects and individuals affected by cerebral neoplasms. (Heritage et al,1981; De Matini et al,1995; Amoli et al,2021) [43-45].

Both JCV and BKV encode two oncoproteins, namely large T antigen (T-Ag) and small t antigen (t-Ag), which play a critical role in the transformation of cells [46]. Binding to the retinoblastoma-associated protein (RB) and p53, BKV Large T-Ag effectively blocks apoptosis [47]. In contrast, JCV T-Ag triggers G2 checkpoint pathways mediated by ataxia-telangiectasia mutated (ATM)/ATM- and Rad3-Related (ATR), leading to the arrest of G2 cell cycle [48]. Additionally, JCV T-Ag causes DNA damage in the host cell, thereby resulting in genetic instability in infected cells [49]. As such, both BK and JC viruses cause chromosomal abnormalities in cells via a 'hit and run' mechanism, which recruits neighboring and distant cell proliferation [34]. Chronic infection is established for both viruses throughout life, and their T-Ag is detected in various types of human cancers, such as breast cancer [50] [51].

In conclusion, the results of the current investigation reveal that the prevalence of BKV virus in breast cancer cases is remarkably low, estimated at around 2%; however, all specimens tested negative for the JC virus genome. Nevertheless, further research efforts are necessary to establish a link between BK virus and breast cancer.

Author Contribution Statement

Alipour Z. drafted the manuscript; Makvandi M. designed the study, performed experimental, and edited the final version of the manuscript; Kayedani GA; suggested the research idea and corrected the manuscript; Talaiezadeh AH acquisition of data; Javadi M. analysis and interpretation of data; Alipour Z,preparation of samples.

Acknowledgements

The authors extend their gratitude to all members of the Department of Laboratory Sciences, School of Allied Medical Sciences, at Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, for their valuable cooperation in facilitating this study.

Ethics consent

The present investigation has obtained approval from the Ethics Committee (IR AJUMS.MEDICINE. REC.1399.039) affiliated with Ahvaz University of Medical Sciences, thereby ensuring the adherence to ethical principles and guidelines.

Funding Statement

This study was carried out by Miss. Zohreh Aalipour MSc(virology) with grant no: CRC-9926 was financially supported by cancer research center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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