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Original Article

Frequency of BK Polyomavirus in Bladder Cancer

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Abstract

Background and Aims: The BK virus a member of the Polyomaviruses family was first isolated from the urine of the kidney recipient. Infection with this virus and infection usually occurs in childhood (5-9 years) but most of the time (90%) of sera are positive and without symptoms. Polyomaviruses including the BK virus have also been suggested to be a contributing factor to some cancers in humans such as brain tumors, bone tumors, Kaposi's sarcoma, adrenal tumors, renal carcinoma, prostate cancer, urinary tumors, genital tumors etc. However, the topic still remains controversial.

Materials and Methods: In this study, we report the presence of BK specific DNA sequences in bladder cancer by PCR and Nested PCR methods.

Results: Our study results confirmed the presence of BK in 13.7% of the samples by Nested PCR method.

Conclusion: In conclusion, 51 samples 13.7% of paraffin-embedded bladder cancer sample were confirmed by Nested PCR method.

Keywords: Seroprevalence; Infectious Laryngotracheitis; Broiler; Iran; ELISA

Introduction

BK Polyomaviruses is a non-enveloped virus with approximately 42 nanometer size whose capsid protects cyclic double-stranded DNA (1). The capsid surface contains 72 capsomers, each of which is composed of four VP1 molecules, the major capsid protein. Capsule is composed of VP2 and VP3 single protein molecules. These virions contain 88% protein and 12% DNA (2). The BK virus is a member of the Polyomaviruses family, first isolated from the urine of the kidney recipient and then named the BK virus. (3) Studies show that infection with the virus causes tissue changes in kidney allografts and then transplant rejection. BK virus is nephrotropic, however its nucleic acid sequences and proteins have been found in other tissues including blood, brain, liver, heart, lungs as well as gonads, etc. (4). Infection with this virus usually occurs in childhood (5-9 years), however, most of the time (90%) the infection remains asymptomatic. After the initial infection, the virus persists in the host body in some cases, when the immune system is suppressed or impair, the virus reactivates and causes symptomatic disease. When activated by the virus in the kidney transplant, the virus replicates more rapidly in the epithelial

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cells and renal tubules, leading to necrosis and loss of kidney function and ultimately transplant rejection (5, 6).

The virus also causes hemorrhagic cystitis, which causes inflammation of the bladder mucosa resulting in dysuria, hematuria, and hemorrhage that can cause clots (7).

BK virus has been suggested to be a contributing factor to some cancers in human (8). A wide range of cancers associated with BK infection including brain tumors, bone tumors, Kaposi's sarcoma, adrenal tumors, renal carcinoma, prostate cancer, urinary tumors, genital tumors, etc (9).

Bladder cancer accounts for 5% of new cancers diagnosed in the United States and it is the sixth most common malignancy. About 90% of patients are over 55 years of age and the mean age at diagnosis is 73 years. The race is supposed to be a predisposing factor it is more than twice as likely to be found in blacks, this malignancy was estimated to cause 16400 deaths in 2016 in the USA (10).

Bladder cancer Transitional Cell Carcinoma is the most common type of bladder cancer which has a high rate (11). In transitional cells, which are capable of expanding and contracting the bladder when it is full, are disrupted non-urothelial bladder cancer, including squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and mixed histologic tumors, squamous cell carcinoma, and adenocarcinoma which include the majority of non-urothelial tumors (12). From another point of view, Bladder cancer is histologically divided into low grade and high-grade disease. While low-grade tumors are generally non-invasive (TA), high-grade tumors can be classified as invasive bladder cancer, as invasive non-muscle bladder cancer (NMIBC) or invasive bladder cancer. Low-grade tumors are associated with a high recurrence rate but a good prognosis and 90% of patients have 5-year survival. In contrast, high-grade NMIBC has a relatively poor 5-year survival (13, 14). Here we report the presence of BK genome sequences in high-grade and low-grade bladder cancerous tissue samples.

Methods

Sample Collection. 51 paraffin embedded formalin fixed samples of bladder cancer diagnosed the past 15 years were collected from pathology tissue bank of university hospital in IRAN. 26 high-grade and 25 low-grade samples were included in this study.

Sample Preparation. Initially, paraffin-embedded formalin fixed (FFPE) tissue blocks were prepared and sliced into 15 to 10 micrometer by microtome, and were placed into sterile microtubes with the minimum of paraffin entering the tube.

That DNA extraction was performed manually according to the following instructions as described before (1):

1. One ml of xylene (Xylen C8H10) was added to the samples and immediately vortexed vigorusly, next kept at room temperature for 15 minutes, then centrifuged at 10,000 rpm, in order to dissolve paraffin.
2. This step was repeated 3 times to remove the remaind paraffins.
3. One ml of 96% ethyl alcohol (C2H5OH) was added to the microtiter and well vortexed, then kept at room temperature for 15 minutes, then centrifuged at 10,000 rpm, the supernatant was completely dissolved.
4. In case of observation turbidity in the microtope, step 1 was repeated once more and then alcohol was added and the other steps were continued as above.
5. Then, a drop of acetone (Acetone) was added to the microtope to eliminate ethyl alcohol.
6. The samples were incubated at 56°C for 24 hours to dry the samples.
7. Then, one ml of digested solution plus 20 micro liter of proteinase K (Fermentase) was added to the micro titer and incubated at 56°C for 24 hours.
8. The microtubes were boiled for 20 minutes and then centrifuged at 10000 RPM to remove cell debries.
9. The supernatant contained DNA

After the above steps, the extracted DNA concentration was calculated using a nanodrop (thermo company), and samples with a concentration above 200 ng / ml and a ratio of 260/ 280 between 1.8 and 2 were frozen at -20 ° for subsequent steps.

PCR amplication. A conserved part of the largT antigen polioma virus (BK) regions was used for amplification and analysis.

Preparation of primers. Internal and External primers were previously used to BK amplication were blasted by nucleotide blast (www. ncbi.com), the two set primers were JCVF1-JCVR1 External primers and BKVF2-BKVR2 primers as internal primers (2).

Specific fragment was amplified using External Specific Primers by PCR reaction in a total volume of 25 µl components of PCR are shown in Table (1).

The temperature programs listed in Table (2) were also used to reproduce by specific external primers.

Nested PCR. Internal fragments were amplified using specific internal primers and 1 µl of PCR product of first run (Table 1) PCR thermal program (protocol) was optimized by thermal gradient analysis and a PCR protocol for both runs was used as shown in Table 2.

Electrophoresis of samples. After Nested PCR, 4 µL of amplication from PCR product was electrophoresed on 1.5% agarose gel and distilled water served as negative control-BK virus DNA was used as positive control.

Result

Blast analysis showed 100% homology of primers with BK T Ag sequences. A 385bp band corresponding to specific amplified fragment was observed in positive samples. The thermal gradiant analysis determined 58°C as the best Tm for all PCR reactions, next Nested round performed to reveal false negative samples and a 278 bp band was observed. Figure (5).

Fig. 1. It shows the 385bp band of the PCR positive response against the ladder.

Fig. 2. The 274bp band shows a positive Nested PCR response against the ladder.

Statistical analysis. In this study 51 samples of bladder cancer (26 high-grade and 25 low-grade) with mean age of 60.7±14.1 were included among 15 females (33.33%) and 30 males (66.66%). In our study, 51 positive samples were obtained from 7 positive samples, of which 3 were high-grade and 4 were low-grade, indicating the presence of BK polyomaviruses in 13.7% of the total samples and and 86.3% of patients were negative for BK virus. Overall, 18% of low grade cancer patients were positive for BK, while 11.5% of high grade patients were positive for BK virus (p=0.703).

Table 1: Quantity of tested materials in PCR.

Materials	Final Concentration	Required Volume (µl)
Sterile distilled water for injection	-	6
PCR master mix	2x	10
External Forward Primer	5 pmol / µl	2/5
Internal Forward Primer	5 pmol / µl	2/5
DNA	200 ng / µl	4
final Volume	-	25

Table 2: Temperature program in PCR steps.

Number of cycles	Temperature (Centigrade)	Time (seconds)	levels
1	94	240	Initial Denaturation
35	94	45	Secondary Denaturation
35	58	60	Early Annealing
35	72	45	Initial Extension
1	72	480	Secondary Extension

Table 3: Quantity of tested materials in Nested PCR.

Materials	**Final concentration**	**Required volume (µl)**
Sterile distilled water for injection	-	9
PCR master mix	2X	10
External Forward Primer	5 pmol / µl	2/5
Internal Forward Primer	5 pmol / µl	2/5
DNA	400 ng / µl	1
final Volume	-	25

Discussion

In recent years, polyomavirus has been suggested to be one of the causative agents of cancer. The early region of the BK genome encodes two viral oncoproteins called large Tag and small tAg that act as active oncogenes (3). In addition, it should not be forgotten that the small t and larg T antigens of these viruses can play an important role in their pathogenesis larg T Ag (LT-Ag) first binds to and inactivates two cellular tumor suppressor proteins, including retinoblastoma (pRb) and p53. pRb controls the cell and inhibits the expression of S-phase genes in the cell cycle, while p53 stops the cell and can lead to apoptosis (4-6). Large T Ag BK virus has different roles that alter normal physiological metabolism of cells by neoplastic transformation and immortalization (7). The major role of T Ag in transformation and oncogenicity is the ability to bind and inhibit p53 tumor suppressor function and the pRB family (P105 RB1, P107 and P130 RB2) (8, 9). When Tag BK virus binds to the p53 protein, it disables its function. The direct clastogenic effect of viral oncoprotein may increase in human cells because it inhibits p53-induced apoptosis and increases survival of DNA-damaged cells, increased likelihood of transcription, and Immortality (10). Small T Ag binds to the catalytic and regulatory subunits of 2A PP2A protein (PP2A) and disables their function (11). PP2A is a serine-threonine phosphatase that regulates the protein kinase-activated phosphorylation signal (12). It has recently been proven to be a tumor suppressor in lung, colon, breast carcinoma and melanoma (13). PP2A binding plays an important role in virus transformation as it is conserved as tumor viruses containing small DNA, thus the small tag and middle tag of the polyoma virus bind to the PP2A. It is not part of the large T Ag, suggesting that PP2A connectivity is a distinct small tag function (14). Separation of large T Ag expression from capsid, virus assembly, and cell lysis may also be involved in the development of BKV-associated urothelial cancers (15). BKPyV and JCPyV antibodies exist in 70%-80% of adults. Viruses appear to be ubiquitous and virtually no region of the world is free of anti-poliomyelitis antibodies, except in some remote areas (16). Initial infections with BKPyV and JCPyV viruses are likely to occur in childhood and appear to be subclinical (17, 18).Viruses are likely to have peripheral blood lymphocytes or can persist in the kidney (19-21). It is hypothesized that human PyVV will probably remain indefinitely in the kidney and re-activated and excreted in the urine during immunodeficiency (22, 23). Immuno-suppression or other viral infections may also lead to reactivation of PyVs (22-25).

BK virus is one of the most famous oncoviruses which is morphological and phenotypic features were found by inactivating pRB and p53 tumor suppressors in bladder tumors, finding a carcinogenic mechanism involving the BKPyV large tumor oncoprotein/ antigen. The pathogenesis of these tumors is unclear, but given the overall gap between graft and tumor growth, the possibility of neoplasms following BKPyV infection may be multifactorial. Other potentially contributing elements include exposure to carcinogens, greater viral mutation, and cell genomic instability in viral integration. A team of researchers identified PCR in normal tissue and tumors from urinary tract BKV patients in 50% of the cases (Mounini et al., 1995b). This number, with the prevalence of BKV in the population, represents 70% of the population with the BK virus. In addition, the urinary tract appears to be a viable place to find the virus, as research has shown that the virus persists in the kidney. It was largely diagnosed, but the virus was absent in any of the normal tissues (26). Transitional Cell Carcinoma (TCC) is the most common type of bladder cancer and approximately 69% of bladder cancers are of this type (27). In this cancer, transitional cells, which are capable of expanding and contracting the bladder when it is full, are disrupted (28). In recent decades, numerous studies have attempted to link the existence of the BK genome and its protein products to human neoplasms, however, numerous studies have remained controversial about the role of BK in human malignancies, although on the basis of non-existent evidence Adequate in humans and ample evidence in laboratory animals WHO has recently classified BK as a possible carcinogen for humans (3). Various carcinogenic and cocarcinogenic factors are involved in bladder cancer, including age, sex, smoking, alcohol use, type of occupation, chemicals, coloring, oil, and long-term use of painkillers and anti-cancer drugs. Cancer, exposure to substances such as benzidine, aromatic polycyclic hydrocarbons, contact with arsenic, water sources, Schistosoma infection, viral infections and a number of unknown chromosomal events have been noted, among which the role of viral infections has been much discussed in upcoming years (29, 30).

Future research on the virus should therefore aim to provide insights into how they change from benign pathogens to persistent viral forms of malignancy or acute diseases such as cystitis, PML, and cancer

In these studies, it would be desirable if a small number of viruses were checked by Real Time-PCR but not financially constrained. Also, the use of several samples as a control would not be possible because of all the paraffin block tumors and the lack of number. Most of the bladder concussion specimens are due to a shortage of postgraduate Master; however the results of our research are reliable results.

Conclusion

In conclusion, 51 samples 13.7% of paraffin-embedded bladder cancer sample were confirmed by Nested PCR method.

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