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STUDY ON HEPATITIS B AND BK VIRUSES IN PATIENTS WITH OSTEOSARCOMA

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Abstract:

Background and Objective*: Osteosarcoma is an unusual bone cancer with high-grade malignancy which generally affects adolescents and young adults. Osteosarcoma is usually treated by preoperative and postoperative chemotherapy and surgery. Several risk factors may involve in development of osteosarcoma cancer, among them the association of BK virus with osteosarcoma have been already investigated. Thus we aimed to determine the association of BK and HBV virus among the patients with osteosarcoma.*

Methods*: Fifteen formalin-fixed paraffin-embedded tissues samples of patients with osteosarcoma including 10 male and 5 female were collected. After DNA extraction from each sample, Polymerase Chain Reaction (PCR) was performed for detection of BK DNA virus and Hepatitis B DNA Virus (HBV).*

Results*: Three out of fifteen (20%) participants consisting of 2(13.3%) male and (6.66%) female showed positive for BK virus by PCR. All the 3 patients were high grade primary osteosarcoma with mean age of 13 to 19 year old. NO HBVDNA was detected in the patients with osteosarcoma.*

Conclusion*: Although high prevalence of BK, about 20%, was observed among the patients with osteosarcoma, more comprehensive studies are needed. To manage and improve treatment the screening of BK virus DNA should be implemented for all the osteosarcoma patients.*

Keywords: *Osteosarcoma, BK virus, Hepatitis B Virus, Polymerase Chain Reaction*

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INTRODUCTION:

Osteosarcoma is a bone tumor characterized by the production of osteoid by malignant cells. The disease onset can occur at any age; however primary high-grade osteosarcoma occurs most commonly in the second half of life span. Parosteal osteosarcoma has a peak incidence in the third and fourth decades, and secondary osteosarcoma is more common in older individuals. The incidence is a slightly higher in males. All of the skeletal systems can be affected; however, most of primary osteosarcoma occurs at the sites of the most rapid bone growth, including the distal femur, the proximal tibia, and proximal humerus. Almost all patients with high-grade osteosarcoma report progressive pain. The average delay form the onset of symptoms to the correct diagnosis was approximately 15 weeks. This period can be divided into the delay for patient to notify anomalies of the disorder, about 6 weeks, and delay for physician diagnosis which is about 9 weeks. Plain radiographs are the most valuable tools for making the correct diagnosis. Magnetic resonance imaging (MRI) is the best modality to measure the extent of the tumor within the bone and in the soft tissue. A bone scan should be obtained to look for skeletal metastases, and radiography and computed tomography (CT) of the chest should be performed to search for pulmonary metastases; the lungs are the most common site of metastases. The tests should be done before biopsy (29).

BK polyomavirus (BKPyV) is an important agent of polyomavirus-associated nephropathy (PyVAN) and polyomavirus-associated haemorrhagic cystitis (PyVHC) (1). The genome of polyomavirus BK (BKV) is about 5 kbp, double-stranded, circular DNA molecule with three different regions: the non-coding control region (NCCR) comprises the origin of DNA replication and promoter/enhancer for early and late transcriptions. The regulatory proteins, large T and small t antigens are encoded by the early region. The late region encodes the structural proteins VP1, VP2, VP3 (2, 3). BKV transmission occur during early childhood and It have been estimated 90% of adult individuals have antibodies against BKV worldwide (4, 5). Primary infection BKV is asymptomatic and persists latently in the kidney of immunocompetent individuals (6). Reactivation takes place in immune suppressed conditions, such as patients receiving organ transplants and patients with HIV infection (7, 8, 9). Intracerebral and intravenous inoculation of BKV in newborn hamsters induced malignant tumors (mainly ependymomas, malignant insulinomas, and osteosarcomas)(10). Osteosarcoma is high-grade primary skeletal malignancy, very heterogeneous genetic profile and primary malignancy of bone in children and young adults. Characterized by spindle cells of mesenchymal origin depositing immature osteoid matrix (11). Osteosarcoma comprises roughly 5% of childhood cancers and 20% of all bone tumors (12). Treatment including chemotherapy and surgical techniques, which result in cure in 60%–75% of patients (13), about one-third of young patients recur or develop metastatic disease or metastasis to the lungs. Survival for patients with metastatic disease is <20% (14) There has been little improvement in survival during the last 20 years, and new therapeutic approaches are needed. The detection of BKV DNA has been reported in 78% of osteosarcomas and 38% of Ewing's tumors (15, 16).

Hepatitis B virus (HBV) is a DNA virus belongs to hepadnaviridae family, responsible for chronic, acute, and fulminant hepatitis, which are prevalent worldwide. Chronic HBV may lead to cirrhosis and hepatocellular carcinoma (17, 18). The association of HBV and hematologic malignancies, including Hodgkin lymphoma and non-Hodgkin lymphoma (NHL) has been previously studied (19). Detection of preS-binding on SaOS-2 (osteosarcoma cell line) has been investigated (20). Therefore, the present study was aimed to evaluate BKDNA and HBVDNA in block samples of patients

The aim of this study was to determine the BHDNA and HBV DNA among block samples of patients with osteosarcoma in Ahvaz city, Iran. All procedures of this study were approved by the local research ethic committee of Ahvaz Jundishpur University of Medical Sciences (Reg. code: IR.AJUMS. REC.1396.94).

MATERIALS AND METHODS:

Bone osteosarcoma is the most common bone cancer after the multiple myeloma cancer. To our knowledge, no study has been conducted during 2013-1026, on the osteosarcoma suspected patients to study the association between the HBV and BKV and the occurrence of the osteosarcoma tumor. In this study, the patients referred to the orthopedic clinic were examined for clinical symptoms, MRI scan, Bone scan, and age and finally 15 patients (age range=9 to 19 years old) diagnosed with bone osteosarcoma were selected for the study. The patients underwent standard biopsy process and after the confirmation of the disease by pathologist, the samples were referred to the virology department for molecular and cellular assessments to determine the association of HBV and BKV with the disorder.

Paraffin embedded tissues of 15 block formalin-fixed paraffin-embedded tissues samples including 10 (66.66%) males and 5 (33.33%) females patients were collected from Imam Khomeini hospital located in Ahvaz, Iran, during 2012 to 2016. The

diagnosis of laryngeal cancer and tumor grade was approved by a pathologist. The following steps were then carried out for the detection of BKDNA and HBV DNA in the patient samples.

DNA Extraction

The sections of 5 µm thickness were prepared from each sample. Deparaffinization was done with xylene and ethanol (Germany, Merck). Initially, all the fragments specimens were placed in Microtubes; then, Xylene was added and kept at 45 °C for 15 min followed by centrifuge at 14,000rpm. This stage was repeated again. Then, the supernatant was discarded and 1ml absolute ethanol was added to precipitate and stored at the room temperature for 10 min and centrifuged at 14,000rpm for one minute. The supernatant was discarded. This process was repeated by adding 70% ethanol, same as the previous steps. Finally, supernatant was discarded and microtubes were placed at 65C° for 5 min to vaporize the ethanol residue.

Then, 200 µl lysate buffer and 50 µl proteinase was added to each microtube. The microtube was then vertex and incubated at water bath at 55 °C for one week. Every day microtubes were vertex and kept again in water bath. Then tubes were centrifuged at 14000rpm for 5 min. The supernatant was collected and utilized for DNA extraction. The DNA extraction of all the tissue samples was carried out using the high pure PCR template kit (Roche, Germany), according to the manufacturer's instructions.

Detection of BKVDNA by PCR

Primers used to amplify the typing region were Forward primer 5'- C AAG TGC CAA AAC TAC TAA T-3' and Reverse primer 5'- CAT GAA GGT TAA GCA TGC -3' (21).

The extracted DNA was amplified using 5 µL DNA template, 0.3 µL Taq Polymerase, 1 µL dNTP, 0.25 µL each forward and reveres primers, 2 µL $MgCl^2$ and 5 µL 10X PCR buffer, all mixed in a microtube and the ultimate volume reach to 50 µL using nuclease-free distilled water. For each run, we used Diethylpyrocarbonate (DEPC) water as a negative control and confirmed positive sample DEPC water as a negative control and confirmed positive sample from our previous study (9) as a positive control. After activation of reaction in 95°C for 5 minutes, amplification was per-formed for 50 cycles. Cycle program was as: 94°C for 60, 55°C for 60 and 72°C for 90 seconds. A final extension 72°C for 5 minutes was the ultimate step of cycles. Polymerase chain reactions (PCRs) were performed using the Peqlab thermocycler (Peq star, 96 Universal Gradient -Germany). The 342 bp amplification product was detected using 1.7% gel agarose socked in Ethidium bromide (Figure 1). Positive samples for BKV were sent to Noor Genetic Laboratory, Ahvaz, Iran for sequencing. Sequences were analyzed using the neighbor Toning phylogenetic tree software and compared by reference ones.

Nested Polymerase Chain Reaction

The nested PCR for detection of HBV DNA was performed for all the tissue samples. The primers for partial sequencing of the "S" region was used ; FHBS1: 5′-GAG TCT AGA CTC GTG GTG GAC TTC-3′ (position 244 – 267), and RHBS1:5′-CGT GGT GGA CTT CTC TCA ATT TTC-3′ (position 668 – 691); and inner primers, FHBS2: 5′-AAA TKG CAC TAG TAA ACT GAG CCA-3′ (position 255 - 278), and RHBS2: 5′-GCC ARG AGA AAC GGR CTG AGG CCC-3′ (position 648 – 671) (22). For the first round, 5 µL of the extracted DNA from each sample was added to a PCR reaction mixture containing 0.5 µL dNTP (10 Mm), 2.5 µL PCR buffer (10x), 0.15 µL 5U Taq DNA polymerase (Roch, Germany), 50 pmol/µL of the FHBS1 and RHBS1 primers, 0.5 µL dNTP (10 Mm) and 15.85 µL distilled water. The samples were Placed in the thermal cycler (Techne Company, UK) and the first round amplification was carried out with initial denaturation at 94°C for five minutes, followed by denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 30 seconds, for a total of 30 cycles. For the second round, 5 µL of the PCR product was added to 25 µL of reaction mixture containing the same components mentioned in the first run including dNTP, PCR buffer and Taq DNA polymerase with 50 pmol/µL of each of FHBS2 and RHBS2 primers. Amplification was carried out in the thermal cycler with the same program as the first round. Next, 8 µL of the nested PCR product (417 bp) was analyzed by 2% agarose gel electrophoresis.

RESULTS:

The age of patients was ranged from 9 to 19 years old. The location of osteosarcoma was 3 in proximal bita, and found in 2 male and 1 female. 4 located in Proximal homros and observed in 2 male and 2 in female. 8 found in distal femur, 6 in male and 2 in female.

3/15 (20%) samples including 2 (13.3%) male and (6.66%) female showed positive for BK virus by PCR (Photo 1). NO HBVDNA was detected in the patients with osteosarcoma. Among 3 osteosarcoma positive BKV DNA one of them diagnosed with osteoblastic, pattern, one telangiectatic type and one small cell type osteosarcoma

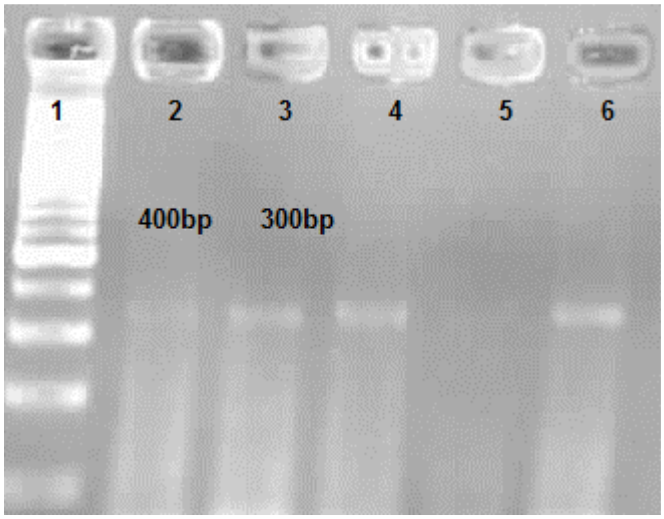

Fig 1: Line 1, Molecular Marker, line 2, 3, 4 positive control, line 5 negative control and 6 , positive control.

DISCUSSION:

Osteosarcoma is categorized as primary or secondary. Primary osteosarcomas are subcategorized as conventional osteosarcoma, low-grade intramedullary osteosarcoma, parosteal osteosarcoma, periosteal osteosarcoma, high grade surface osteosarcoma (23).

Osteosarcoma is a high-grade mass of malignant mesenchymal cells with osteoid production and local tissue invasion. Conventional osteosarcomas are further classified into osteoblastic, chondroblastic, or fibroblastic types, depending on which matrix-producing cells dominate and prognoses (23). Other high-grade central osteosarcomas include telangiectatic, giant cell-rich, small cell, and epithelioid variants, each with characteristic histology and small differences in survival (24).

Human polyomavirus BK (BKV) DNA and proteins have been detected in a number of bone tumors. We have established a persistently BKV-infected cell culture (U-2OS15E) by infecting human osteosarcoma cells. The same proportion of cells that contained episomal BKV genomes expressed both early and late BKV antigens, indicating productive infection (25). In the present study the number of male patients with osteosarcoma was two times of female patient which is in agreement with finding reported by in our present study out of 15 patients with osteosarcoma 3 patients including 2 male and 1 female showed positive for BKVDNA by PCR. All the 3 patients were high grade primary osteosarcoma. Among 3 osteosarcoma positive BKV DNA one diagnosed with osteoblastic, pattern, one telangiectatic type and one small cell type osteosarcoma surprisingly, the age of patient was ranged between 9- 19 years. Osteosarcoma is the most common primary malignancy of bone in children and young adults (26). Although some studies have reported that of BKVDNA have been detected in older patients with primary osteosarcoma (27, 28). Primary high grade osteosarcoma generally takes place in the second decade of the life.Parosteal sarcoma occurs in the third and fourth decades, secondary osteosarcoma are more common in individuals (23, 27, 28, 29). Our experiments reveal that a specific BKV variant is associated with tumour, osteosarcomas.

A recent report by Russo et al. also suggests the role of BKV in the pathogenesis of prostate cancer. The have reported BKV DNA was detected in 85% of the prostate cancer specimens but in none of the benign prostatic hyperplasia control group by PCR (30).

The presence of BKV sequences have been reported in brain tumors, pancreatic tumors, lymphomas, adrenal tumors, colorectal tumors, melanoma, and Kaposi's sarcoma. Since BKV has been detected in peripheral blood cells of normal individuals (31, 32, 33, 34, 35, 36). Monini et al. reported the detection of BKV sequences by PCR and Southern blotting in more than 50% of both normal and tumor tissues obtained from the urinary tract and prostate (37). In addition, viral DNA load was found to be significantly higher in the neoplastic tissues compared to non-neoplastic tissues, suggesting that there may be a selection for BKV-containing tumor cells (37).

Antivirals such as cidofovir and fluoroquinolone are now being tested in cases of BKV-associated disease (38). In the light of aforementioned

description on BK virus and its association with different cancerous cell it is recommended the screening of BKDNA by high sensitivity molecular means such as PCR and real-time PCR should be carried out for all the cancer patients before chemotherapy.

CONCLUSION:

Although high prevalence BKV, about 20%, was observed among the patients with osteosarcoma, more comprehensive studies are needed to reach decisive conclusion. To manage and improve the treatment for osteosarcoma, screening of BK virus DNA should be implemented for all the osteosarcoma patients.

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Footnotes

AUTHORS' CONTRIBUTION:

Mohammad Fakoor was responsible for the accuracy of the data and contributed to the design and performance of the study. Keyan Bahrebar has collected the samples from patients, Manoochehr Makvandi and Gholam Abbas Kaydani and Rahil Nahid samiei participated in the laboratory evaluations and performed the literature review. Manoochehr Makvandi drafted the manuscript and was the guarantor. All authors read and approved the final manuscript.

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REFERENCES:

1- Low J, Humes HD, Szczypka M, Imperiale M**.** 2004. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. Virology **323:**182-188.

2- Van Ghelue M, Khan MT, Ehlers B, Moens **U.** 2012. Genome analysis of the new human polyomaviruses. Reviews in medical virology **22:**354-377.

3- Gosert R, Rinaldo CH, Funk GA, Egli A, Ramos E, Drachenberg CB,Hirsch HH**.** 2008. Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication and cytopathology. J.Exp.Med. **205:**841-852

4. Knowles, W. A. 2006. Discovery and epidemiology of the human polyomaviruses BK virus (BKV) and JC virus (JCV). Adv Exp Med Biol **577:**19-45.

5- Kean, J. M., S. Rao, M. Wang, and R. L. Garcea**.** 2009. Seroepidemiology of human polyomaviruses. PLoS Pathog **5:**e1000363.

6- Low J, Humes HD, Szczypka M, Imperiale M**.** 2004. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. Virology **323:**182-188.

7- Gosert R, Rinaldo CH, Funk GA, Egli A, Ramos E, Drachenberg CB,Hirsch HH. 2008. Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication andcytopathology. J.Exp.Med. **205:**841-852.

8- Reploeg, M. D., G. A. Storch, and D. B. Clifford**.** 2001. Bk virus: a clinical review. Clin Infect 529 Dis **33:**191-202.

9- Sundsfjord, A., Flaegstad, T., Flo, R., Spein, A.R., Pedersen, M., Permin, H., Julsrud, J., Traavik, T., 1994. BK and JC viruses in human immunodeficiency virus type 1-infected persons: prevalence, excretion, viremia, and viral regulatory regions. J. Infect. Dis. 169, 485 490

10-Sekiguchi M, Miyazaki S, Fujikawa K, Shiroko Y, Asanuma K. BK virus-induced osteosarcoma (Os515) as a model of human osteosarcoma. Anticancer Res. 1996; 16 (4A) : 1835-42.

11- Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. Int J Cancer. 2009;125(1):229–34.

12- Skubitz KM, D'Adamo DR. Sarcoma. Mayo Clin Proc. 2007; 82(11):1409–1432.

13-Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, et al. Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. J Clin Oncol. 1997; 15(1):76–84.

14- Ritter J, Bielack SS. Osteosarcoma. Ann Oncol. 2010; 21(Suppl 7):vii320–vii325.

15- De Mattei, M., Martini, F., Corallini, A., Gerosa, M., Scotlandi, K., Carinci, P., Barbanti Brodano, G., Tognon, M., 1995. High incidence of BK virus large-T-antigen-coding sequences in normal human tissues and tumors of different histotypes. Int. J. Cancer 61, 756 760.

16- Diller, L., Kassel, J., Nelson, C.E., Gryka, M.A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S.J., Vogelstein, B., 1990. p53 functions as a cell cycle control protein in osteosarcomas. Mol. Cell Biol. 10, 5772 5781

17-McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology*. 2009;**49**(5 Suppl):S45–55. doi: 10.1002/ hep.22898

18-Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet*. 1999;**353**(9160):1253–7.

19-Marcucci F, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: epidemiology, mechanisms of

tumorigenesis, and therapeutic opportunities. *Blood.* 2011;**117**(6):1792–8.
20- Jung-Hyun Park, Eun-A Choi, Eun-Wie Cho, Yun-Jung Lee,Jung-Min Park, Shin-Young Na, and Kil Lyong Kim. Detection of Cellular Receptors Speci®c for the Hepatitis B Virus preS Surface Protein on Cell Lines of Extrahepatic Origin. Biochemical and Biophysical Research Communications **277,** 246 ±254 (2000)

21- Takasaka T,Goya N,Tokumoto T,Tanabe K,Toma H,Ogawa et al. subtypes of BK virus prevalent in Japan and variant in their transcription region.J Gen Virol.2004;85(Pt 10):2821-7
22-Sitnik R, Pinho JR, Bertolini DA, Bernardini AP, Da Silva LC, Carrilho FJ. Hepatitis B virus genotypes and precore and core mutants in Brazilian patients. *J Clin Microbiol.* 2004; **42**(6):2455–60. doi: 10.1128/JCM.42.6.2455-2460.2004.
23- Fox MG, Trotta BM. Osteosarcoma: review of the various types with emphasis on recent advancements in imaging. Semin Musculoskelet Radiol. 2013;17(2):123–36.
24- Klein MJ, Siegal GP. Osteosarcoma: anatomic and histologic variants. Am J Clin Pathol. 2006;125(4):555–81.
25- Christine Hanssen Rinaldo , Marit Rene´e Myhre , Hilde Alstad , Øivind Nilssen ,
Terje Traavik.Human polyomavirus BK (BKV) transiently transforms and persistently infects cultured osteosarcoma cells. Virus Research 93 (2003) 181-187
26-Gurney JG, Severson RK, Davis S, Robison LL. Incidence of can-cer in children in the United States. Sex-, race-, and 1-year age-spe-cific rates by histologic type. Cancer 1995; 75: 2186-95.
27- Dae Geun Jeon, Soo Yong Lee, Wan Hyung Cho, Won Seok Song Jong Hoon Park. Primary Osteosarcoma in Patients Older than 40 Years of Age *J Korean Med Sci 2006; 21: 715-8*
28- Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer. 2009;115(7):1531–43.
29- S.Terry Canal, James H.Beaty. Campbell,s operative orthopaedics. [12] ed, Vol.1 ,ELSEVIER,Mosby.(2013) pp 909-45
30- Russo G, Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Giordano A, et al. p53 gene mutational rate, Gleason score, and BK virus infection in prostate adenocarcinoma: is there a correlation? J Med Virol 2008;80(12):2100–7.
31- Corallini A, Pagnani M, Viadana P, Silini E, Mottes M, Milanesi G, et al. Association
of BK virus with human brain tumors and tumors of pancreatic islets. Int J Cancer 1987;39(1):60–7
32- Engels EA, Rollison DE, Hartge P, Baris D, Cerhan JR, Severson RK, et al. Antibodies
to JC and BK viruses among persons with non-Hodgkin lymphoma. Int J Cancer 2005; 117 (6):1013–9.
33- Barzon L, Trevisan M, Masi G, Pacenti M, Sinigaglia A, Macchi V, et al. Detection
of polyomaviruses and herpesviruses in human adrenal tumors. Oncogene,2008;27(6):857–64.
34- Casini B, Borgese L, Del Nonno F, Galati G, Izzo L, Caputo M, et al. Presence and incidence of DNA sequences of human polyomaviruses BKV and JCV in colorectal tumor tissues. Anticancer Res 2005;25(2A):1079–85.
35- Giraud G, Ramqvist T, Ragnarsson-Olding B, Dalianis T. DNA fromBK virus and JC virus and from KI,WU, and MC polyomaviruses aswell as from simian virus 40 is not detected in non-UV-light-associated primarymalignant melanomas of mucous membranes. J Clin Microbiol 2008;46(11):3595–8.
36- Monini P, Rotola A, de Lellis L, Corallini A, Secchiero P, Albini A, et al. Latent BK virus infection and Kaposi's sarcoma pathogenesis. Int J Cancer 1996;66(6):717–22.
37-Monini P, Rotola A, Di Luca D, De Lellis L, Chiari E, Corallini A, et al. DNA rear-rangements impairing BK virus productive infection in urinary tract tumors. Virology 1995;214(1):273–9.
38 - Rinaldo CH, Hirsch HH. Antivirals for the treatment of polyomavirus BK replication. Expert Rev Anti Infect Ther 2007;5(1):105–15.