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ORIGINAL ARTICLE

Do polyomavirus hominis strains BK and JC play a role in oral squamous cell carcinoma?

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Abstract

Introduction. Head and neck cancers are the most common cancers worldwide. It is estimated that approximately 90% of all head and neck cancers are represented by squamous cell carcinoma (SCC). There are many risk factors causing this type of cancer, including environmental factors and lifestyle choices, such as tobacco smoking or abusing alcohol. Other important risk factor include infectious factors.

Objective. The aim of this study was to analyze the prevalence of BK and JC virus infections among patients with oral squamous cell carcinoma (OSCC).

Materials and method. The correlation between BKV infection and OSCC, and correlation between BKV, OSCC, alcohol abuse, tobacco smoking, demographic data, pre-treatment staging, metastases of lymph node evidence, and grading, was analyzed. The study group consisted of 92 patients with squamous cell carcinoma (OSCC), 75 males, and 17 females, aged between 40 – 87 (average 56.8). All the patients underwent surgery and were not subjected to chemotherapy or radiotherapy prior to treatment. The analyzed samples were collected from paraffin sections.

Results. BKV DNA was detected in 18.5% of patients with OSCC. In the control group, BKV DNA was detected in 3.3%. BKV DNA was statistically more frequently detected among patients with squamous carcinoma, compared to the control group ($p<0.05$).

Conclusions. The obtained results suggest that the BKV virus may play an important role in the development of oral squamous cell carcinoma.

Key words

BK virus, oral squamous cell carcinoma, OSCC

INTRODUCTION

The BK virus (BKV, polyomavirus hominis, BKPyV,) is a small, non-enveloped, circular double-stranded DNA virus, belonging to the *Polyomaviridae* family. The name of this virus derives from the initials of the patient (BK) from whom it was first isolated. The *Polyomaviridae* family contains 2 mammalian genera: *Orthopolyomavirus* and *Wukipolyomavirus*. The *Orthopolyomavirus* genus includes the JC virus (JCPyV), BK virus (BKPyV), Simian virus SV40, Merkel cell polyomavirus (MCV, MCPyV), and trichodysplasia spinulosa-associated polyomavirus. The *Wukipolyomavirus* include the KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), and Human polyomavirus -6,-7,-9 [1]. It is estimated that up to 90% of the general population may be BKV infected, mainly during childhood (between 5–10 years of age). The transmission route is not clearly defined; however, the oro-faecal and respiratory route transmission are the most feasible as the upper respiratory tissues are more susceptible to infections. Additionally, BKV-induced nephropathy is a well-known problem among kidney transplant recipients [2, 3, 4, 5, 6]. What is more, the BKV DNA has been detected in human brain tumours, neuroblastoma, urinary tract tumours, carcinomas of the uterine cervix, vulva, lips and tongue, and in Kaposi's sarcoma. Correlation between BKV and metastatic bladder carcinoma among immunosuppressed transplant recipients, and among BKV and prostate and bladder carcinoma, was analyzed. This is probably in consequence of the kidneys being the main site of BKV latency [6].

On the other hand, salivary glands have been described as a potential location of the virus, as the presence of BKV genetic material was detected in saliva [7]. The BKV virus is detected in the oral cavity, and probably has tropism to squamous cells.

Head and neck cancers (consisting of tumours localized in the lips, oral cavity, nose and para-nasal sinuses, nasopharynx, oropharynx, hypopharynx, and larynx [8]) are the most common cancers worldwide. It is estimated that approximately 90% of all head and neck cancers are squamous cell carcinomas (SCC) [9].

There are many risk factors causing this type of cancer as it is associated with environmental factors as well as lifestyle choices, such as tobacco smoking or abusing alcohol. Other important risk factors include infectious factors, such as human papillomavirus (HPV) [9, 10]. HPV infection has been reported in up to 50% of head and neck squamous cell carcinomas [11]. The role of other viruses in the development of cancer is still being researched. One such virus is the BK virus. Apart from the above factors, deficiency of vitamin A, riboflavin and iron, poor oral hygiene, and immunosuppressive therapy, are also correlated with a higher incidence of oral carcinoma [12].

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Objective. The aim of the study was to analyze the prevalence of BK and JC virus infection among patients with oral squamous cell carcinoma.

MATERIALS AND METHOD

Tissue specimens were obtained from 92 patients with OSCC operated on between 1995–2005 at the Department of Otolaryngology, Head, and Neck Surgery of the Medical University in Lublin, Poland. The study group consisted of 75 males and 17 females aged between 40–87 (average 56.8). The samples were collected from formalin-fixed and paraffin-embedded tumour-tissue. The specimens were collected during the first diagnosis when TNM (T-tumour, N-nodus, M- metastasis) was diagnosed by examination. According to clinical diagnosis, the patients were classified as follows: 11 cases – II°, 18 – III°, and 63 – IV°. Patients were not subjected to chemotherapy or radiotherapy prior to surgery. TNM classification was performed in accordance with the UICC (Union Against Cancer) criteria. Histological grading was performed in accordance with the World Health Organization (WHO) criteria, which divides tumours into well-differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3). The epidemiological, clinical and pathological characteristics are shown in Table 1.

The control group consisted of 30 healthy volunteers, from whom oral mucosa swabs containing epithelial cells were collected. It was impossible to obtain a sample of oral cavity tissue as such a procedure would not be accepted by the Ethics Committee.

The research was approved by the Ethics Committee and conducted in accordance with the GCP regulations (No. KE-0254/181/2012).

DNA extraction. DNA was extracted from 3, 10-µm thick sections of formalin-fixed and paraffin-embedded tissue. The samples were transferred to Eppendorf tubes after cutting deep into the block. The microtome blade was changed each time. 1,000 µl of xylene was added to test-tubes, mixed using puls-vortexing, and later centrifuged (3 min, 8,000 rpm). Then the supernatant was removed. 1 ml of 96% ethanol was added to remove the residual xylene. The samples were vortexed and centrifuged at 800 rpm for 3 minutes. Supernatant was carefully removed. The opened tubes were incubated at 37°C until the residual ethanol evaporated.

DNA measuring. Measurement of DNA concentration was performed using spectrophotometry, and the β-globin gene amplified to evaluate the DNA extraction process and the presence of amplification reaction inhibitors.

Control samples. DNA from the urine of a kidney transplant patient was used as a positive PCR control to assess the success of amplification (ATCC VR-837). PCR reagents without template DNA served as a negative control.

PCR. A polymerase chain reaction (PCR) method was used to detect the BK/JC virus in specimens. For detection of the genetic material of the BK/JC virus, the primers described for the first time by Arthur et al. were used: PEP-1 (5'-AGTCTTTAGGGTCTTCTACC-3') and PEP-2 (5'-GGTGCCAACCTATGGAACAG-3'). [13]. The oligonucleotides attach to a highly conservative region of early coding T-Ag. Because of the high homology of BKV and JCV genomes (75%), polymers are complimentary to the DNA of both viruses. The described primer pair can

Table 1. Epidemiological. clinical and pathological features of the study group

Epidemiological, clinical and pathological features		BKV positive			BKV negative		statistical significance
		total	n=17	%	n=75	%	
Gender	female	17	4	23.5	13	76.5	χ^2=0.35 P=0.55
	male	75	13	17.3	62	82.7	
Age	40–49	23	6	26.1	17	73.9	χ^2=3.9 P=0.14
	50–60	40	9	22.5	31	77.5	
	>60	29	2	6.9	27	93.1	
Place of residence	big city	27	6	22.2	21	77.8	χ^2=0.36 P=0.84
	small city	29	5	17.2	24	82.8	
	rural	36	6	16.7	30	83.3	
Smoking	yes	79	14	17.7	65	82.3	χ^2=0.26 P=0.88
	no	5	1	20	4	80	
	no answer	8	2	25	6	75	
How long/in years	<20	12	3	25	9	75	χ^2=1.44 P=0.7
	>20	34	7	20.6	27	79.4	
	no answer	42	7	16.7	35	83.3	
	no smoking	4	0	0	4	100	
How many/per ay	<10	8	3	37.5	5	62.5	χ^2=4.91 P=0.17
	>10	63	9	14.3	54	85.7	
	no answer	17	5	29.4	12	70.6	
	no smoking	4	0	0	4	100	
Alcohol abuse	Yes	57	12	21.1	45	78.9	χ^2=0.67 P=0.71
	No	6	1	16.7	5	83.3	
	no answer	29	4	13.8	25	86.2	
General diseases	No	57	12	21.1	45	78.9	χ^2=4.77 P=0.44
	Circulation	18	2	11.1	16	88.9	
	Diabetes	3	0	0	3	100	
	COPD	11	0	0	11	100	
	Others	9	3	33.3	6	66.7	
Histology stage G	1	14	1	7.1	13	92.9	χ^2=1.62 P=0.65
	2	74	15	20.3	59	79.7	
	3	4	1	25	3	75	
T stage	1	0	0	0	0	0	χ^2=1.02 P=0.6
	2	30	6	20	24	80	
	3	19	2	10.5	17	89.5	
	4	43	9	20.9	34	79.1	
N stage	1	18	3	16.7	15	83.3	χ^2=1.31 P=0.73
	2	53	11	20.8	42	79.2	
	3	3	1	33.3	2	66.7	
	0	18	2	11.1	16	88.9	
M stage	0	91	16	17.8	74	82.2	χ^2=4.67 P=0.1
	1	1	1	0	1	100	
Clinical stage	I	0	0	0	0	0	χ^2=0.84 P=0.66
	II	11	2	18.2	9	81.8	
	III	18	2	11.1	16	88.9	
	IV	63	13	20.6	50	79.4	

therefore be used for detecting both BK and JC viruses. The PCR product sequence is specific for a given virus. Primers amplify a 176-bp fragment of BKV genetic material, and 173-bp fragment of JCV genetic material. Final concentrations of the PCR reaction mixture were: 2.0 mM $MgCl_2$, 200μM dNTPs, 0.25 μM of each primer, 0.5U Hot Start Taq DNA polymerase (Qiagen). Amplification was performed under the following conditions: initial denaturation at 94 °C for 15 min., followed by 40 cycles: 94 °C for 1 min., 55 °C – 1 min., 72 °C – 1 min.; final extension: 72 °C – 10 min.

During each PCR run, samples were tested, together with one negative and one positive control. The PCR products were analyzed using electrophoresis in 2% agarose gel, and then purified using a Gel-Out kit (A&A Biotechnology) for further analysis.

PCR product sequencing. Purified PCR products were sent to a commercial sequencing facility (Genomed Company) to confirm the results using direct sequencing because of the existing homology between polyomaviruses BK virus, and JCV. Typing was performed using BLAST algorithm (basic Local Alignment Search Tool; http://blast.ncbi.nlm.gov/Blast.cgi). The sequences were used in order to construct a phylogenetic trees. Phylogeny was based on the maximum likelihood method (ML), which requires the use of computer programess such as PAUP 4.0, ModelTest 3.7, PhyML 2.4.4 and MEGA 4.1. In order to choose the appropriate model of molecular evolution, hLRTs (hierarchical Likelihood-Ratio Test) and AIC (Akaike Information Criterion) tests were used. The reference strains sequences used in the study was taken from the public database GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

RESULTS

BKV DNA was detected in 18.5% of patients with OSCC, whereas the JCV was not detected at all. In the control group, BKV DNA was detected in one sample which constituted 3.3%. BKV DNA was statistically more frequently detected among patients with squamous carcinoma in comparison to control group ($p<0.05$). In the BKV-infected group there were 4 females and 13 males. The majority of the tumours were G2: 74 (80.4%), G1:14 (15.2%), and G3:4 (4.4%). In the study group, there were no patients classified as T1, 30 (32.6%) were classified as T2, 19 (20.7%) – T3 and 43 (46.7%) – T4. N0 18 (19.6), N1 53 (57.6), N2 3 (3.2), N3 18 (19.6). Among BKV-positive patients there were 17.7% non-smokers and 29.4% non-drinkers (among BKV-negative 13.3% and 40%, respectively).

Although there was no statistical significance, it cannot be excluded that infectious factors play an important role among non-smokers and non-drinkers. Squamous cell carcinoma is most frequent in males (81.5%), smokers (85.9) and drinkers (61.9). 73.9% were over 50 years of age. There are also very interesting results concerning patients younger than 50. In this age group, there were 35.3% of BKV-positive and 22.7% of BKV-negative patients. This result may suggest that infection plays an essential role, especially among younger patients. The most important results are shown in Table 2. The relationship between various strains of the BK virus identified in the Polish patients is present on the phylogenetic tree- dendrogram (Fig. 1). The majority of BKV strains sequences were the same genotype (58%).

Table 2. The most important results of the study group

Study group	BKV positive	BKV negative	statistical significance
patients with OSCC	18.5%	81.5%	P<0.05
control group	3.3%	96.7%	
non-smokers	17.7%	13.3%	
non-drinkers	29.4%	40%	
patients younger than 50	35.3%	22.7%	

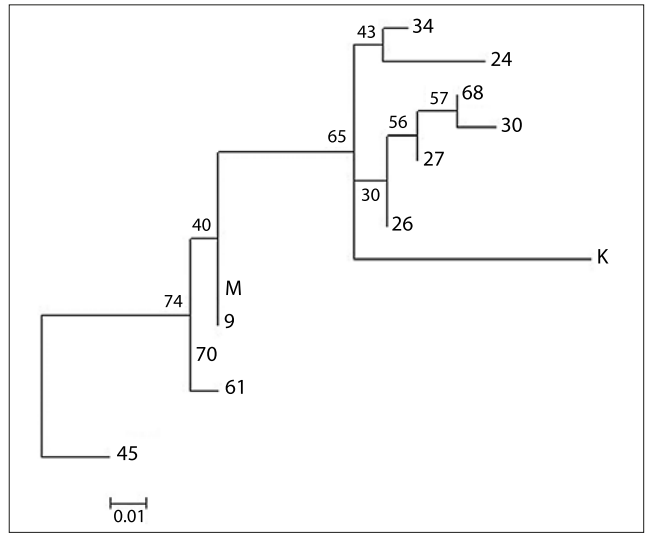

Figure 1. Phylogenetic analysis of BKV strains identified in the Polish patients. Analysis is based on a 176 bp sequence in the T-Ag region of BKV genome

DISCUSSION

Oral cancer is a serious problem for public health. It is the eleventh most common malignancy in the world, and squamous cell carcinoma (SCC) is a predominant histological type [12]. Squamous cell carcinoma (SCC) accounts for 5 – 10% of all new cancer cases in the USA and Europe, and in 2008 there were 500,000 cases of SCC recorded worldwide.

The etiology of oral squamous cell carcinoma is considered to be multifactorial and risk factors include tobacco smoking and alcohol abuse [9,10], which is also confirmed by the results of the presented study. The study group consisted of 85.7% smokers (among BKV-negative patients 86.7%, and among BKV-positive patients – 82.3%), and 62% drinkers (70.6% and 60%, respectively). Other important risk factor includes infectious agents (oncogenic viruses).

The role of HPV (*Human papillomavirus*) has already been established, and the correlation between human papillomavirus infection and HNSCC (head and neck squamous cell carcinoma) was suggested back in 1983, and confirmed a few years later [8, 15]; therefore, the presented study focused on other viruses from this oncogenic virus group. BK virus is widespread among general, healthy adults, and according to different authors, approximately 60–80% – 90% of adults exhibit specific antibodies. This virus remains in a latent stage and can reactivate under immunosuppressive conditions due to BKV-associated nephropathy [16, 17]. Recent data suggests a correlation between the BK virus and various types of human cancers: Kaposi's sarcoma, brain tumours, and tumours of the urinary tract [16].

The role of BKV in oral squamous cell carcinoma is controversial. However, to the best of the knowledge of the authors of the current article, only one research focused on BKV and oral squamous cell carcinoma negates the correlation between the two. Some authors suggest that the BK virus may be a potential co-factor for HPV in the development of cervical neoplasia [19], especially together with the HPV genotype 16 [20]. What is more, BKV DNA presence was confirmed in high-grade squamous intraepithelial cervical lesions (pre-cancerous lesions) [20].

In the light of the presented research results, and the opinions of other researchers, the role of BKV in oral SCC cannot be excluded, taking into account that the genetic material of BKV was detected in saliva [7].

It is realized that formalin-fixed and paraffin-embedded tumour tissue research is problematic, not only because of the existence of many inhibitors, but also because of the more serious problem of tissue cutting during which viral DNA is also cut, and as a result it may not be detected in other samples.

Squamous cell carcinoma is most frequently observed among individuals aged over 50 (in this research, 73.9% of the participants were over 50). However, some data prove that this problem is becoming more and more frequent among younger individuals [21]. In this study, it was observed that 35.3% of BKV-positive and 22.7% BKV-negative patients were within the age range 40–49. These results may suggest that infections play an important role among then younger group of patients whose exposure to alcohol and tobacco smoking was generally shorter than in the older group.

CONCLUSIONS

The above obtained results suggest that BKV may play an important role in the development of oral squamous cell carcinoma. The study does not confirm the importance of the JC virus in cancer development.

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