ACTA MICROBIOLOGICA BULGARICA Volume 34 / 1 (2018)

Prevalence of Human Polyomaviruses KIPyV and MCPyV in Respiratory Tract Specimens from Bulgarian Patients

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Abstract

The role of KI polyomavirus (KIPyV) and Merkel cell polyomavirus (MCPyV) as human pathogens is not completely understood. The aim of this study was to determine the prevalence of these polyomaviruses in patients with respiratory diseases (acute and chronic, including lung cancer). 221 nasopharyngeal swabs and lung biopsy specimens were studied for the presence of KIPyV and MCPyV by nested PCR. KIPyV and MCPyV were detected in nasopharyngeal specimens at similar rates, 10.3% and 8.2%, respectively. We found 2.8% KIPyV positivity rate among patients with non-cancer chronic lung diseases. All specimens from lung cancer cases were negative for KIPyV and MCPyV. The results suggested that there may be a relationship between these polyomaviruses and acute respiratory diseases, but no evidence was found for their involvement in lung cancerogenesis.

Keywords: KI polyomavirus, Merkel cell polyomavirus, prevalence, PCR, respiratory diseases, lung cancer

Резюме

Ролята на KI полиомавирусите (KIPyV) и на свързаните с Меркел-клетъчния карцином полиомавируси (MCPyV) като човешки патогени не е напълно изяснена. Целта на настоящото проучване е да се установи разпространението на тези полиомавируси при пациенти с респираторни заболявания (остри и хронични, в т.ч. и рак на белия дроб). Изследвани са 221 назофарингеални смивове (НФС) и проби от биопсичен материал от бял дроб (БМБ) за наличие на KIPyV и MCPyV чрез нестед PCR. Не установихме съществени различия в разпространението на KIPyV и MCPyV в НФС - открити бяха съответно в 10.3% и 8.2% от случаите. 2,8% от БМБ, получени от пациенти с хронични неонкологични белодробни заболявания бяха положителни за KIPyV. Всички проби от случаите с рак на белия дроб бяха отрицателни за KIPyV и MCPyV. Резултатите предполагат възможна връзка между тези полиомавируси и острите респираторни заболявания, но липсва доказателство за тяхното участие в белодробната канцерогенеза.

Introduction

The first human polyomaviruses (HPyV), JC polyomavirus (JCPyV) and BK polyomavirus (BKPyV), were discovered in 1971 in severely immunosuppressed patients. Today, due to the new techniques for viral detection, the human polyomavirus family consists of thirteen members (Table 1).

KI polyomavirus (KIPyV) and Merkel cell polyomavirus (MCPyV) are among the newly (2007-2008) identified HPyV (Allander *et al.,* 2007; Feng *et al.*, 2008). KIPyV was named after the Karolinska Institute (Stockholm, Sweden), where the virus was discovered and MCPyV - due to its detection in Merkel cell carcinoma (MCC). Although

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Table 1. Human polyomaviruses (HPyV)

Full name	Abbreviation	Year of discovery	Source of isolation
BK polyomavirus	BKPyV	1971	Urine
JC polyomavirus	JCPyV	1971	Urine, brain
Karolinska Institute polyomavirus	KIPyV	2007	Nasopharyngeal tissue
Washington University polyomavirus	WUPyV	2007	Nasopharyngeal tissue
Merkel cell polyomavirus	MCPyV	2008	Lesion
Human polyomavirus 6	HPyV6	2010	Skin
Human polyomavirus 7	HPyV7	2010	Skin
Trichodysplasia spinulosa-associated polyomavirus	TSPyV	2010	Lesion
Human polyomavirus 9	HPyV9	2011	Skin, blood, urine
Malawi polyomavirus	MWPyV	2012	Stool, wart
St Louis polyomavirus	STLPyV	2013	Stool
Human polyomavirus 12	HPyV12	2013	Metastatic liver tissue
New Jersey polyomavirus	NJPyV-2013	2014	Endothelial cells (myositis, cut. necrosis)

KIPyV and MCPyV share certain characteristics with the well-studied BKPyV and JCPyV, they show important differences such as little sequence homology of genomes and proteins, differences in viral life cycle, pathogenicity and so on (Dalianis and Hirsch, 2013; Feltkamp *et al.,* 2013). Little is known about the route of infection, transmission, cell tropism and pathogenicity of these viruses.

KIPyV was identified first in respiratory specimens from children with acute respiratory infections, which suggested the respiratory tract as a possible site of infection, transient or persistent (Allander *et al.*, 2007). So far, however, the etiopathogenicity of KIPyV in respiratory diseases remains speculative. To date, KIPyV has not been associated with a specific disease and data on its oncogenicity are controversial (Babakir-Mina *et al.*, 2009; Dalianis *et al.,* 2009). Although viral DNA sequences have been found in lung cancers and lymphoma, KIPyV has not been linked to a specific malignancy (Babakir-Mina *et al.*, 2011).

MCPyV has been implicated in the etiology of MCC but most MCPyV infections are asymptomatic. MCPyV DNA has been detected in a variety of non-MCC cancers, including lung cancer (Andres *et al.*, 2010; Imajoh *et al.*, 2012; Spurgeon and Lambert, 2013; Salakova *et al.*, 2015), but the role of MCPyV in these malignances is not clear.

There has been intensive search for these viruses in clinical specimens to determine their natural history and role in human pathology, but the results are inconclusive. Therefore, we aimed to evaluate the distribution of KIPyV and MCPyV in Bulgarian patients with respiratory diseases – acute and chronic, including lung cancer, in order to evaluate the association between these virus infections and respiratory diseases.

Material and Methods

Clinical samples

We analyzed 133 nasopharyngeal swabs (NPS) and 88 lung biopsy specimens (LBS) obtained from 107 males and 114 females with respiratory diseases aged between 1 and 83 years. NPS were from patients with acute respiratory diseases of higher and lower respiratory tract (pharyngitis, tonsillitis, common cold, bronchitis, pneumonia, etc.) and LBS - from patients with chronic lung diseases (53 histologically proven lung cancer cases and 35 cases with non-cancer chronic lung diseases).

Detection of KIPyV and MCPyV by nested PCR

DNA extraction was performed by GeneJET Genomic DNA Purification Kit (Thermo Scientific™) and PureLink® Genomic DNA Mini Kit (Invitrogen™). We assessed the quality of extracted DNA by β- globin gene amplification. Detection of KIPyV and MCPyV was performed by nested

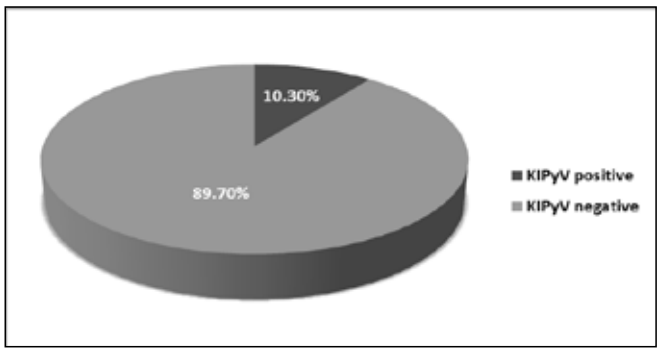

Fig. 1. KIPyV prevalence in NPS

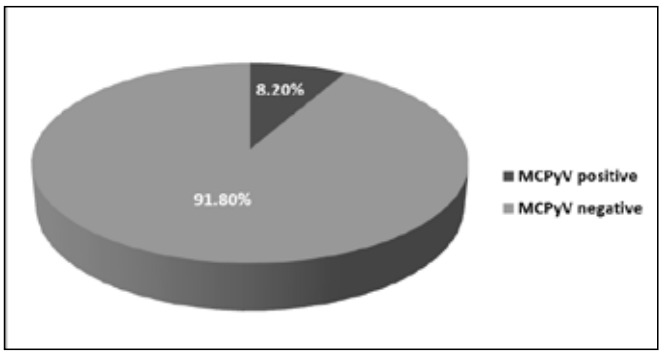

Fig. 2. MCPyV prevalence in NPS

PCR using the following primer sequences: for amplification of KIPyV - 5′-aaggccaagtcaagttc-3′ and 5′-acactcactaacttgatttgg-3′ (first PCR), 5′-cgcagtaccactgtcagaagaaac-3′ and 5′-ttctgccaggctgtaacatac-3′ (second PCR); for amplification of MCPyV - 5’-tgcaaatccagaggttctcc-3’ and 5’-aaaacacccaaaaggcaatg-3’(first PCR), 5’-atattgcctcccacatctgc-3’ and 5’-tgccctaatgttgcctcagt-3’(second PCR). Amplifications were performed on a DNA Engine Opticon 2 system (MJ Research) with 15 μl reaction volume and PCR conditions as follows: denaturation for 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C and final extension step of 7 min at 72°C.

The plasmids containing the complete genome of KIPyV or MCPyV were used as positive controls and distilled water as negative control. The PCR products were analyzed in 2% agarose gels by electrophoresis.

All specimens that were positive for KIPyV and MCPyV were retested.

Statistical analysis

Statistical analysis was performed with SPSS for Windows v.10.0 using Fisher exact test to calculate P values. P values of less than 0.05 were considered statistically significant.

Results and Discussion

Among all 133 NPS, 97 were β-globin positive and were further tested for the presence of KIPyV and MCPyV DNA. Amplifications of 211-bp and 307-bp fragments in the second PCRs were observed in KIPyV and MCPyV positive specimens, respectively. KIPyV and MCPyV prevalence was at similar rates, 10.3% (10 of 97) and 8.2% (8 of 97), respectively (Fig. 1 and Fig. 2).

NPS were obtained from patients with acute respiratory diseases of the upper and lower respiratory tract (pharyngitis, tonsillitis, common cold, bronchitis, pneumonia, etc.). All KIPyV positive samples were from patients with flu-like symptoms. Positive for MCPyV were patients with flu-like symptoms (5), pneumonia (2) and with tonsillopharyngitis (1). In our study we found higher KIPyV (10.3%) and MCPyV (8.2%) prevalence in NPS compared with results from other countries (KIPyV: 0.5% to 8%; MCPyV: 1.3-4.2%) (Bialasiewicz *et al.*, 2009; Goh *et al.*, 2009; Kantola *et al.*, 2009; Kiassari et al., 2011; Babakir-Mina *et al.*, 2013; Iaria *et al.*, 2015).

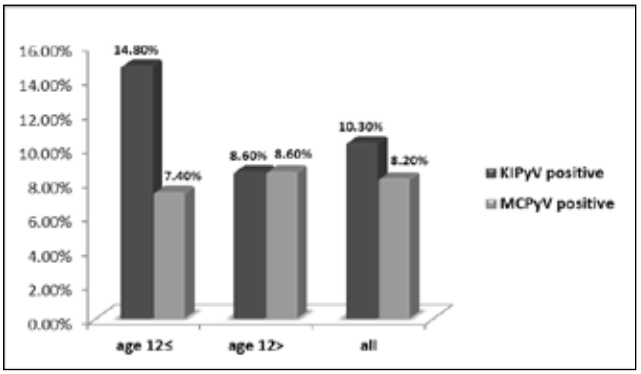

Fig. 3. KIPyV and MCPyV prevalence according to age

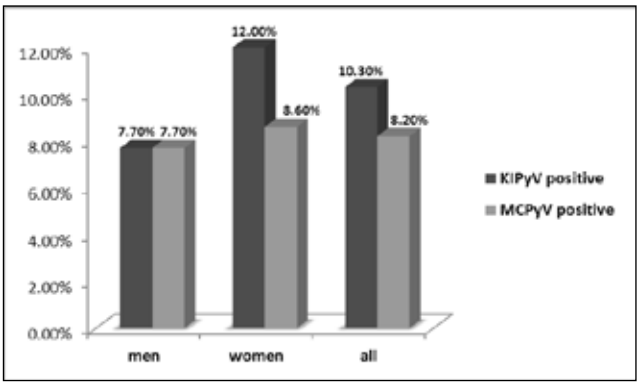

Fig. 4. KIPyV and MCPyV prevalence according to gender

We analyzed the presence of KIPyV and MCPyV in NPS obtained from the following groups of patients: men and women;"aged 12 years or less" and "older than 12 years". The results showed that the difference of the KIPyV and MCPyV status according to the age and gender was not statistically significant (Fig. 3 and Fig. 4).

Previous studies concerning KIPyV or MCPyV involvement in lung cancerogenesis were controversial. Some of them have shown presence of these polyomaviruses in the lung tissue and lung cancer (Anders *et al.*, 2009; Babakir-Mina *et al.*, 2009; Gheit *et al.*, 2012; Kim *et al.*, 2017). Therefore, we tested 53 LBS collected from patients with histologically proven lung cancer. 35 specimens from cases with non-cancer chronic lung diseases (pulmonary fibrosis, hamartoma, COPD, inflammatory pseudotumors, sarcoidosis) were used as negative controls. All lung cancer specimens were KIPyV and MCPyV negative. Similar results were observed by others studies not able to detect KIPyV and MCPyV in lung cancer tissues (Wetzels *et al.*, 2009; Teramoto *et al.*, 2011; Karami *et al.*, 2014). Of the 35 tested samples from patients with non-cancer chronic lung diseases only one (2.8%) was KIPyV positive. It was obtained from a patient with hamartoma.

Conclusions

The high KIPyV and MCPyV prevalence found in specimens from patients with acute respiratory diseases in this study suggested a potential role of KIPyV and MCPyV infection in the development of these diseases. KIPyV and MCPyV were not detected in lung cancer samples, indicating no oncogenic potential and involvement of these viruses in lung cancerogenesis.

Acknowledgements

This work was supported by the Bulgarian National Science Fund (Grant No ДКОСТ 01/1) and by COST Action BM1201.

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