SHORT REPORT

Detection of four human polyomaviruses (MCPyV, HPyV6, HPyV7 and TSPyV) in cervical specimens from HIV-infected and HIV-uninfected women

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ABSTRACT

Objectives To investigate the presence of recently discovered human polyomaviruses in cervical specimens collected from African and French women, in relation to HIV serostatus, high-risk human papillomavirus infection (HR-HPV) and cervical disease.

Methods Cervical specimens were collected from 140 HIV-1-seropositive African women and 50 HIV-seronegative French women. Presence of Merkel cell polyomavirus (MCPyV), human polyomavirus 6 (HPyV6), human polyomavirus 7 (HPyV7) and trichodysplasia spinulosa-associated polyomavirus (TSPyV) was detected by real-time PCR, and presence of HR-HPV DNA by Hybrid Capture 2 assay with subsequent HPV genotyping using the INNO-LiPA HPV Genotyping Extra assay. Cervical biopsies were analysed by histopathology.

Results The detection rates were 55.3%, 3.2%, 2.1% and 0% for MCPyV, HPyV6, HPyV7 and TSPyV, respectively, with no significant difference by population. The MCPyV viral load ranged from 14 to 210 DNA copies/10^6 cells (median, 80 DNA copies/10^6 cells), with no difference between women with and without cervical precancerous lesions. There was no association between detection of human polyomaviruses in cervical specimens and geographical origin/HIV serostatus, HR-HPV coinfection or precancerous cervical lesions.

Conclusions These observations argue against a possible role of MCPyV as a cofactor in HPV-induced carcinogenesis. MCPyV and, to a lesser extent, HPyV6 and HPyV7 might belong to the female genital tract microbiota.

INTRODUCTION

Cervical cancer is the third most common female cancer worldwide and the fourth leading cause of cancer death in women. Persistent infection of the uterine cervix with oncogenic high-risk types of human papillomavirus (HR-HPV), particularly HPV16, is recognised as the cause for the development of precancerous cervical lesions that may progress to invasive cancer.[1] However, only a minority of women infected with HR-HPV develops premalignant and malignant lesions, suggesting that cofactors are involved in HR-HPV-associated carcinogenesis.

Polyomaviridae is a continuously growing family of viruses with up to 11 new human representatives recently added to the previously described BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV).[2] Human polyomaviruses (HPyVs) subclinically infect the general population and may induce disease in immunocompromised patients.[2] Moreover, these viruses have the potential capacity to induce tumours through their tumour antigen (T-Ag) oncoproteins.[2] The oncogenic capabilities of HPyVs were recently illustrated with the discovery of the Merkel cell polyomavirus (MCPyV), which is now commonly accepted as the aetiological agent of the Merkel cell carcinoma, a rare but aggressive skin cancer.[3]

Several studies have investigated the presence of HPyVs in different tissues, and their presence in the female genital tract may suggest an association between HPyVs and precancerous or cancerous cervical lesions, as illustrated by the detection of BKPyV, JCPyV and MCPyV genomic sequences in cervical cancer cases.[4–7]

In order to study more deeply the relationships between HPyVs, cervical lesions and immune status, we have investigated the presence of recently described HPyVs including MCPyV, HPyV6, HPyV7 and the trichodysplasia spinulosa-associated polyomavirus (TSPyV) in cervical samples from African and French women, in relation to HR-HPV coinfection, cervical disease stage and HIV serostatus.

METHODS

A total of 190 cervical samples collected with the Digene cervical sampler and placed in the specimen transport medium (STM) (Qiagen, Gaithersburg, Maryland, USA) were analysed. One hundred and forty samples were collected from a subset of women with a median age of 35.4 years (range, 21–47 years) enrolled in the HARP (HPV in Africa Research Partnership) study, which is conducted in two Sub-Saharan African countries, South Africa and Burkina Faso, with the aim to evaluate cervical cancer screening approaches in HIV-1 infected African women. In addition, 50 samples were collected from randomly selected HIV-seronegative women with a median age of 40.0 years (range, 25–68 years) attending a gynaecological outpatient department at the Montpellier University Hospital, France, who were tested for HR-HPV infection. Cervical biopsies were obtained from all of the

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African women who had a positive HR-HPV test and/or abnormal cervical cytology and/or abnormal visual inspection using acetic acid and Lugol's iodine, and these women were selected to provide similar proportions of women with high-grade cervical intraepithelial neoplasia 2 or higher (CIN2+) and women with normal histological findings or low-grade lesions (≤CIN1). In the group of French women, biopsies were performed only in case of abnormal cytological findings.

Written informed consent was obtained from the women. Ethical approval was granted by the Ministry of Health in Burkina Faso (no 2012-12-089), the Witwatersrand University in South Africa (no 110707) and the London School of Hygiene and Tropical Medicine (no 7400).

HR-HPV DNA was detected using the Hybrid Capture 2 assay (Qiagen) with subsequent HPV genotyping using the INNO-LiPA HPV Genotyping Extra assay (Fujirebio, Les Ulis, France).

DNA was extracted from STM using the NucliSens EasyMag extraction kit (Biomérieux, Craponne, France) with the NucliSens EasyMag automated extractor. Detection of MCPyV, HPyV6, HPyV7 and TSPyV DNA was performed by real-time PCR using specific primers. Quantification of MCPyVs DNA was normalised to the amount of whole genomic DNA as previously described.[8] For the other viruses, semiquantification was based on the cycle threshold (Ct) value and samples with Ct>40 were considered as negative.

Proportions were compared using the χ^2 test or Fisher's exact test, and the MCPyV DNA levels were compared using the Mann–Whitney U test.

RESULTS

HR-HPV DNA was detected in cervical specimens from 124 (88.6%) HIV-1-positive African women and from 24 (48.0%) HIV-negative French women. HPV16 DNA was detected in cervical specimens from 36 (27.8%) African women and 6 (12.0%) French women. CIN2+ lesions were found in 65% (91/140) of the selected African women and 2% (1/50) of the French women.

Overall, MCPyV, HPyV6, HPyV7 and TSPyV DNA was detected in 105 (55.3%), 6 (3.2%), 4 (2.1%) and 0 (0%) samples, respectively. MCPyV DNA loads were low, with a median MCPyV copy number of 80 copies/10^6 cells (range 14–210 copies/10^6 cells), the lower limit of detection being 10 copies/10^6 cells. Median MCPyV DNA load was 79 copies/10^6 cells in women without CIN2+ lesions and 83 copies/10^6 cells in women with CIN2+ lesions (p=0.75). HPyV6 and HPyV7 DNA levels were always weakly detected in the positive samples, with Cts ranging from 34 to 40.

As shown in table 1, there was no association between the detection of MCPyV DNA and HIV serostatus, the detection of HR-HPV, or the presence of CIN2+ cervical lesions. HPyV6 (n=6) and HPyV7 (n=4) were detected in 4 and 4 HIV-infected African women and 2 and 0 HIV-negative French women, respectively (p=1.00). The small sample size did not allow for sufficient statistical power to validly analyse the association of HPyV6 and HPyV7 detection with HR-HPV infection and cervical lesions.

DISCUSSION

This study involved two very different populations of women in terms of geographic origin, HIV status, prevalence of HR-HPV infection and precancerous cervical lesions. Overall, MCPyV DNA was frequently detected at low levels in cervical specimens, whereas HPyV6 and HPyV7 were rarely detected, and TSPyV was not detected. MCPyV DNA detection was not associated with HIV status, HR-HPV infection or precancerous cervical lesions. Moreover, MCPyV DNA levels were not different between women with or without precancerous lesions. The sample size of HPyV6/HPyV7 was too small for useful statistical comparisons.

These results, which do not support a role of these HPyVs in the development of cervical lesions, are in agreement with a recent study reporting a high frequency of MCPyV detection in cervical carcinomas but no statistically significant association between MCPyV infection and cervical cancer.[7] These observations argue against a possible role of MCPyV as a cofactor of HPV-induced carcinogenesis. Interestingly, the detection rates of MCPyV, HPyV6, HPyV7 and TSPyV in cervical specimens observed in this study are similar to those reported in normal skin specimens, these viruses being considered as belonging to the skin microbiota.[9 10] This observation suggests that MCPyV and, to a lesser extent, HPyV6 and HPyV7 might belong to the

Table 1 Associations between MCPyV detection and geographic origin/HIV serostatus, HR-HPV infection and cervical lesions

Parameter	MCPyV status		OR (95% CI)	p Value
	Positive (N=105) n (%)	Negative (N=85) n (%)		
HIV				
Positive* (n=140)	79 (56.4)	61 (43.6)	1.20 (0.60 to 2.40)	0.588
Negative (n=50)	26 (52.0)	24 (48.0)		
HR-HPV				
Positive (n=148)	81 (54.7)	67 (45.2)	0.91 (0.43 to 1.91)	0.781
Negative (n=42)	24 (57.1)	18 (42.9)		
HPV16				
Positive (n=45)	20 (44.4)	25 (55.6)	0.56 (0.27 to 1.17)	0.095
Negative (n=145)	85 (58.6)	60 (41.4)		
Cervical histology				
CIN2+ (n=92)	54 (58.7)	38 (41.3)	1.31 (0.71 to 2.42)	0.356
≤CIN1 (n=98)	51 (52.1)	47 (47.9)		

CIN2+, cervical intraepithelial neoplasia grade 2 or more severe.
*All African women were HIV-1-positive and all French women were HIV-negative.
HR-HPV, high-risk human papillomavirus infection.

female genital tract microbiota. The transmission routes of these viruses and their mode of propagation to the female genital tract remain to be elucidated, and the hypothesis of genital mucosa contamination by skin viruses via hands or sexual activity cannot be excluded.

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Contributors MS, VF and PK-D contributed to the conception and design of the study. PK-D, JN, NB, BS and HK contributed to the acquisition of data. MS, VF, PK-D, HK and PM contributed to analysis and interpretation of data. BS and SD-M supervised the study in Burkina-Faso and South Africa, respectively. MS, VF, PK-D and PM drafted the manuscript. JN, NB, BS, SD-M and HK were involved in critical revision of the manuscript.

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Competing interests None declared.

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